Carbapenemases: the Versatile β-Lactamases

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INTRODUCTION

Carbapenemases represent the most versatile family of β-lactamases, with a breadth of spectrum unrivaled by other β-lactam-hydrolyzing enzymes. Although known as "carbapenemases," many of these enzymes recognize almost all hydrolyzable β-lactams, and most are resilient against inhibition by all commercially viable β-lactamase inhibitors (114, 150, 225). Some investigators have preferred the nomenclature "carbapenem-hydrolyzing enzymes" to the term "carbapenemases," suggesting that carbapenems are but one segment of their substrate spectrum (182). However, the term carbapenemase has become entrenched in the β-lactamase literature and is used throughout this review.

Carbapenemases belong to two major molecular families, distinguished by the hydrolytic mechanism at the active site. The first carbapenemases described were from gram-positive bacilli. Unlike other β-lactamases known at that time, these enzymes were inhibited by EDTA, thereby establishing them as metalloenzymes. Later work has shown that all metallo-carbapenemases contain at least one zinc atom at the active site that serves to facilitate hydrolysis of a bicyclic β-lactam ring (46). In the mid- to late 1980s, another set of carbapenem-hydrolyzing enzymes emerged among the *Enterobacteriaceae* (134), but EDTA did not inhibit their activity (183). Subsequent studies showed that these enzymes utilized serine at their active sites and were inactivated by the β-lactamase inhibitors clavulanic acid and tazobactam (183, 243).

Until the early 1990s, all carbapenemases were described as species-specific, chromosomally encoded β-lactamases, each with a well-defined set of characteristics. However, the identi-

fication of plasmid-encoded IMP-1, a metallo-β-lactamase in *Pseudomonas aeruginosa* (228), ARI-1 (OXA-23), a class D carbapenemase in *Acinetobacter baumannii* (157, 191), and KPC-1, a class A carbapenemase in *Klebsiella pneumoniae* (245), has changed the patterns of carbapenemase dissemination. What was once considered to be a problem of clonal spread has now become a global problem of interspecies dispersion. Because of the proliferation of new members of established carbapenemase families, it is even more important to try to understand the properties of these enzymes, with all their strengths and limitations. Several excellent carbapenemase reviews have appeared recently, including detailed compilations of the kinetic characteristics of these enzymes (112, 150, 221, 225). Therefore, this review focuses on updated information on the epidemiological and biochemical characteristics of both metallo- and serine carbapenemases.

CLASSIFICATION SCHEMES

Classification of β-lactamases can be defined according to two properties, functional and molecular. In the early work with β-lactamases, before genes were routinely cloned and sequenced, a new β-lactamase was analyzed biochemically by isolating the protein and determining its isoelectric point, followed by enzymatic studies to determine substrate hydrolysis and inhibition characteristics (185, 202). The relative rates of hydrolysis for a broad spectrum of β-lactam substrates, and inhibitor profiles, allowed for the classification of the new β-lactamase. This functional classification process evolved over many years into a widely accepted scheme currently dividing the known β-lactamases into four major functional groups (groups 1 to 4), with multiple subgroups under group 2 that are differentiated according to group-specific substrate or inhibitor profiles (22). In this functional classification scheme, carbapenemases are found primarily in groups 2f and 3.

Classification based on amino acid homology has resulted in

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TABLE 1. Classification of class B metallo-β-lactamases: subclass designations of representative metallo-β-lactamases and consensus sequences based on structural similarities to identified Zn^{2+} ligands[a]

β-Lactamase	Substrate(s)	Ligand(s)[b]					
		Zn1			Zn2		
		116	118	196	120/121	221	263
Subclass B1 (BcII, IMP-1, CcrA, VIM-2, SPM-1)	Broad spectrum	His	His	His	Asp/(Arg/Cys)	Cys	His
Subclass B2[c] (CphA, Sfh-1)	Preferential hydrolysis of carbapenems	(Asn)	(His)	(His)	Asp/(Arg)	Cys	His
Subclass B3 (L1, FEZ-1, Gob-1, CAU-1)	Preferential hydrolysis of cephalosporins	His/Gln	His	His	Asp/His[d]	(Ser)	His

[a] Data are from references 46, 50, and 51.
[b] Amino acids in parentheses do not appear to be active-site zinc ligands.
[c] Zn1 ligand for subclass B2 is inhibitory to enzymatic activity. The amino acids indicated have not been confirmed crystallographically to bind this Zn^{2+} atom.
[d] His121 replaces Cys221 as the second zinc ligand.

four major classes (5, 76, 84), which correlate well with the functional scheme but lack the detail concerning the enzymatic activity of the enzyme. Molecular classes A, C, and D include the β-lactamases with serine at their active site, whereas molecular class B β-lactamases are all metalloenzymes with an active-site zinc. Carbapenemases, β-lactamases with catalytic efficiencies for carbapenem hydrolysis, resulting in elevated carbapenem MICs, include enzymes from classes A, B, and D.

Functional classification schemes that included carbapenemases were first proposed by Bush in 1988 (21). A number of subclassification schemes for the metallo-β-lactamases have subsequently been proposed over the past 10 years. Rasmussen and Bush in 1997 suggested that the functional group 3 metalloenzymes (22) could be divided into three functional subgroups, based primarily on substrate specificities (182), whereas molecular subclasses have been proposed by Frere and colleagues for a number of years (46, 50, 51). In addition to these proposals, other modifications have been suggested for the classification of the metallo-β-lactamases (60). These enzymes are currently divided into three subclasses based on a combination of structural features, zinc affinities for the two binding sites, and hydrolysis characteristics. A consensus scheme is shown in Table 1. Subclasses B1 and B3, divided by amino acid homology, bind two zinc atoms for optimal hydrolysis, while enzymes in subclass B2 are inhibited when a second zinc is bound. Subclass B2 also differs in hydrolysis spectrum, as it preferentially hydrolyzes carbapenems, in contrast to the broad hydrolysis spectrum observed for B1 and B3 enzymes (46).

MOLECULAR CLASS A CARBAPENEMASES

Class A serine carbapenemases of functional group 2f have appeared sporadically in clinical isolates since their first discovery over 20 years ago (134). These β-lactamases have been detected in *Enterobacter cloacae*, *Serratia marcescens*, and *Klebsiella* spp. as single isolates or in small outbreaks (134, 149, 243). Bacteria expressing these enzymes are characterized by reduced susceptibility to imipenem, but MICs can range from mildly elevated (e.g., imipenem MICs of ≤4 μg/ml) to fully resistant. These β-lactamases, therefore, may go unrecognized following routine susceptibility testing.

Three major families of class A serine carbapenemases include the NMC/IMI, SME, and KPC enzymes. Their hydrolytic mechanism requires an active-site serine at position 70 in the Ambler numbering system for class A β-lactamases (6). All have the ability to hydrolyze a broad variety of β-lactams, including carbapenems, cephalosporins, penicillins, and aztreonam, and all are inhibited by clavulanate and tazobactam, placing them in the group 2f functional subgroup of β-lactamases. A fourth member of this class, the GES β-lactamases, was originally identified as an ESBL family, but over time variants were discovered that had low, but measurable, imipenem hydrolysis. This subgroup of GES enzymes is also classified as functional group 2f carbapenemases. Table 2 shows the geographical and chronological detection of the class A carbapenemases.

Chromosomally Encoded Enzymes: SME, NMC, and IMI

The antibiotic resistance profile of strains expressing the chromosomal group 2f β-lactamases is distinctive: carbapenem resistance coupled with susceptibility to extended-spectrum cephalosporins. SME-1 (for "*Serratia marcescens* enzyme") was first detected in England from two *S. marcescens* isolates that were collected in 1982 (146, 243). The SME-1 β-lactamase, along with the nearly identical SME-2 and SME-3, has been found sporadically throughout the United States (49, 178, 179, 208). Infections caused by SME-producing *S. marcescens* infections were found as single incidents or small clusters of up to 19 isolates. SME-producing *S. marcescens* isolates from different geographical locations are not identical, as defined by pulsed-field gel electrophoresis (PFGE), although there may be some degree of clonal spread when several isolates are collected from one location (178, 208).

The IMI (for "imipenem-hydrolyzing β-lactamase") and NMC-A (for "not metalloenzyme carbapenemase") enzymes have been detected in rare clinical isolates of *E. cloacae* in the United States, France, and Argentina (149, 175, 181, 183). NMC-A and IMI-1 have 97% amino acid identity and are related to SME-1, with approximately 70% amino acid identity (146, 183). They all contain the conserved active-site motifs S-X-X-K, S-D-N, and K-T-G of the class A β-lactamases. In addition, these carbapenemases have conserved cysteine

TABLE 2. Emergence of class A carbapenemases

β-Lactamase	Yr	Location	Organism	No. of isolates	Gene location[a]	pI[b]	GenBank accession no.	Reference(s)
SME-1	1982	London, United Kingdom	*S. marcescens*	2	Chrom	9.7	U60295[c]	146, 199, 243
	1985	Minnesota	*S. marcescens*	1	Chrom	9.5		179, 199
	1999	Illinois	*S. marcescens*	2	NR	8.5		49
SME-2	1992	California	*S. marcescens*	5	Chrom	9.5	AF275256	179
	1994–1999	Massachusetts	*S. marcescens*	19	Chrom	9.5		179
SME-3	2003	Illinois	*S. marcescens*	2	Chrom	9.5	AY584237	178
IMI-1	1984	California	*E. cloacae*	2	Chrom	7.05	U50278	183
IMI-2	2001	Hangzhou, China	*E. cloacae*	1	Plasmid	8.1	AY780889	249
NMC-A	1990	Paris, France	*E. cloacae*	1	Chrom	6.9	Z21956	143, 149
	2000	Buenos Aires, Argentina	*E. cloacae*	1	NR	6.9	AJ536087	181
	2003	Washington	*E. cloacae*	1	NR	6.9		175
KPC-1	1996	North Carolina	*K. pneumoniae*	1	Plasmid	6.7	AF297554	245
KPC-2	1998–1999	Maryland	*K. pneumoniae*	4	Plasmid	6.9	AY034847	138
KPC-2	1998	Maryland	*Salmonella enterica* serotype Cubana	1	Plasmid	6.7	AF481906	137
	1998	New York	*K. oxytoca*	1	Plasmid	6.7	AY210887	246
	1997–2001	New York	*K. pneumoniae*	18	NR	6.7		13
			K. oxytoca	1	NR	6.7		
	2001	Massachusetts	*Enterobacter spp.*	4	Plasmid	6.9		74
	2001–2003	New York	*E. cloacae*	1	NR	6.7		14
			E. aerogenes	1	NR	6.7		
	2002–2003	New York	*K. pneumoniae*	29	NR	NR		15
	2003–2004	New York	*K. pneumoniae*	95	NR	NR		17
	2004	New York	*K. pneumoniae*	59	NR	6.7		16
	2004	Zhejiang, China	*K. pneumoniae*	1	Plasmid	6.7	DQ897687	229
	2005	Paris, France	*K. pneumoniae*	1	Plasmid	6.8		145
	2005	Medellin, Colombia	*K. pneumoniae*	2	Plasmid	7.0	DQ523564	217
	2005	New York	*K. pneumoniae*	3	NR	NR		118
	2005	Israel	*E. coli*	4	Plasmid	6.7		147
	2006	Medellin, Colombia	*P. aeruginosa*	3	Plasmid/Chrom	6.8		216
	2006	Medellin, Colombia	*C. freundii*	1	Plasmid	6.8		216
KPC-3	2000–2001	New York	*K. pneumoniae*	24	Plasmid	6.5	AF395881	233
	2003	New York	*E. cloacae*	1	NR	6.7	AY522950	14
	2004	New York	*K. pneumoniae*	3	NR	6.7		16
KPC-4	2004	Scotland	*Enterobacter spp.*	1	NR	NR	AY700571	None (unpublished)
GES-2	2000	South Africa	*P. aeruginosa*	1	Plasmid	5.8	AF326355	174
	2000	South Africa	*P. aeruginosa*	8	Plasmid	5.8		173
	2004	South Africa	*P. aeruginosa*	51	NR	NR		230
GES-4	2002	Japan	*K. pneumoniae*	1	Plasmid	6.9	AB11620	219, 220
GES-5	2004	Athens, Greece	*E. coli*	1	Plasmid	5.8	AY494717	218
	2004	Korea	*K. pneumoniae*	6	Plasmid	5.8		86
GES-6	2004	Athens, Greece	*K. pneumoniae*	1	Plasmid	6.9	AY494718	218

[a] Chrom, chromosome; NR, not reported.
[b] NR, not reported.
[c] Two sequences differing by a single amino acid, Z28968 and U60295, for the *S. marcescens* S6 carbapenemase are reported in the GenBank database. Structural analysis and further sequencing confirmed U60295 as the correct sequence for the SME-1 enzyme (179, 199).

residues at positions 69 and 238 that form a disulfide bridge. The genes for these three β-lactamases are all chromosomally located, with no evidence of mobile element association, a fact that may have contributed to their rarity. More recently, however, genes encoding IMI-2 β-lactamases were found on plasmids in *Enterobacter asburiae* from United States rivers and on a plasmid from an *E. cloacae* isolate from China (9, 249).

Biochemical characterization of purified SME, NMC, and IMI enzymes revealed a broad hydrolysis spectrum that includes the penicillins, early cephalosporins, aztreonam, and carbapenems (Tables 3 and 4). Imipenem hydrolysis was easily measurable, with k_{cat} values of >30 s^{-1}. Meropenem had lower k_{cat} and K_m values than did imipenem. Cefoxitin and extended-spectrum cephalosporins were inefficiently hydrolyzed, when hydrolysis could be detected at all, and cefotaxime was hydrolyzed faster than ceftazidime (124, 179, 183).

These chromosomal β-lactamases can be induced in response to imipenem and cefoxitin. Sequencing of the regions upstream of NMC-A, IMI-1, and SME-1 revealed the presence of a divergently transcribed gene related to the LysR family of DNA-binding transcriptional regulatory genes (143, 146, 183). Deletions within the *nmcR* gene eliminated the inducibility of NMC-A and reduced carbapenem MICs, defining a role for this protein as a positive regulator of NMC-A expression (143). The ImiR protein is 97% identical to NmcR, but an analysis of its regulatory effects has not been published. SmeR is 69% identical to NmcR but has weaker activity as a positive regulator of the *bla*$_{SME-1}$ gene.

Two crystal structures of NMC-A, one native and the other complexed with a penicillanic acid inhibitor, and a structure for SME-1 all show that the overall structure and catalytic residues are similar to those of other class A β-lactamases (139, 199, 201). For NMC-A, the structure of the enzyme did not change conformation when the inhibitor was bound. The disulfide bond between positions 69 and 238 is located near the active site, a feature common among the class A carbapenemases. This disulfide bond is necessary for hydrolytic activity, not just for imipenem hydrolysis, suggesting that it is required to stabilize the enzyme structurally (123, 199).

The two most prominent distinctions between the class A TEM-1 structure and NMC-A are a 1-Å alteration of the position of Asn132, which enlarges the substrate-binding cavity, and a change in conformation of the S3 strand between residues 237 and 240, which increases access to the active site. However, in the crystal structure of SME-1, the position of Asn132 was almost identical to the Asn132 residue position of TEM-1 (199). The major difference in the SME-1 structure compared to TEM and NMC-A was crowding of Ser70 and Glu166 in the active-site cleft, leaving no room for a catalytic water molecule. It is possible that conformational rearrangements upon substrate binding may allow a catalytic water molecule into this position. The structure of SME-1 complexed with a β-lactam molecule would test this hypothesis.

In structure/function studies using site-directed mutagenesis, the ability of the SME-1 β-lactamase to hydrolyze imipenem could not be attributed to a single amino acid residue (122). A substitution of alanine for serine at position 237 reduced imipenem k_{cat} values from 100 s^{-1} to 20 s^{-1}; hydrolysis of cephalothin was also reduced fivefold, but the benzylpenicillin k_{cat} values remained unchanged (200). It seems likely that the important conserved residues of the group 2f carbapenemases together contribute to form an active site that can accommodate and hydrolyze the carbapenems.

TABLE 3. Steady-state kinetic parameters for representative class A carbapenemases[a]

Substrate	SME-1				NMC-A				IMI-1				KPC-2				GES-4			
	k_{cat} (s^{-1})	Rel k_{cat} (%)	K_m (µM)	k_{cat}/K_m	k_{cat} (s^{-1})	Rel k_{cat} (%)	K_m (µM)	k_{cat}/K_m	k_{cat} (s^{-1})	Rel k_{cat} (%)	K_m (µM)	k_{cat}/K_m	k_{cat} (s^{-1})	Rel k_{cat} (%)	K_m (µM)	k_{cat}/K_m	k_{cat} (s^{-1})	Rel k_{cat} (%)	K_m (µM)	k_{cat}/K_m
Cephaloridine	980	100	770	1.3	2,820	100	185	15	2,000	100	1,070	1.9	530	100	510	1.0	490	100	2,200	0.22
Benzylpenicillin	19	1.9	17	1.1	260	9.2	28	9.3	36	1.8	64	0.56	63	12	30	2.1	130	27	160	0.81
Imipenem	104	11	202	0.51	1,040	37	92	11	89	4.5	170	0.52	31	5.8	90	0.34	0.38	0.078	4.7	0.081
Meropenem	8.9	0.91	13	0.68	12	0.43	4.4	2.7	10	0.5	26	0.38	3.6	0.68	13	0.28	NA	NA	NA	NA
Ceftazidime	<0.07	<0.01	ND	ND	4.7	0.17	90	0.052	0.0068	0.00034	270	0.000025	0.49	0.092	230	0.0021	2.5	0.51	1,500	0.0017
Cefotaxime	<0.98	<0.10	ND	ND	286	10	956	0.30	0.30	0.017	190	0.0018	17	3.2	100	0.17	17	3.4	700	0.024
Cefepime	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	12	2.3	540	0.022	NA	NA	NA	NA
Cefoxitin	<0.15	<0.02	ND	ND	5.0	0.18	93	0.053	0.0067	0.015	45	0.00067	0.95	0.18	140	0.0068	85	17	810	0.11
Aztreonam	108	11	260	0.42	707	25	125	5.7	51	2.6	93	0.55	66	12	420	0.16	NDH	ND	ND	ND

[a] References: SME-1, 179; NMC-A, 124; IMI-1, 183; KPC-2, 246; GES-4, 219. Abbreviations: Rel k_{cat}, k_{cat} relative to that of cephaloridine; ND, not determinable; NA, no data available; NDH, no detectable hydrolysis.

TABLE 4. Substrate and inhibition profiles of the carbapenemases

Molecular class	Functional group	Enzyme	Hydrolysis profile[a]					Inhibition profile[b]		Reference(s)
			Penicillins	Early cephalosporins	Extended-spectrum cephalosporins	Aztreonam	Carbapenems	EDTA	Clavulanic acid	
A	2f	NMC	+	+	+	+	+	−	+	124
		IMI	+	+	+	+	+	−	+	183
		SME	+	+	±	+	+	−	+	179
		KPC	+	+	+	+	+	−	+	4
		GES	+	+	+	−	±	−	+	174, 219
B1	3	IMP	+	+	+	−	+	+	−	224
		VIM	+	+	+	−	+	+	−	224
		GIM	+	+	+	−	+	+	−	224
		SPM	+	+	+	−	+	+	−	224
D	2d	OXA	+	+	±	−	±	−	±	225

[a] Symbols: +, strong hydrolysis (generally, k_{cat} of >2 s^{-1}); ±, weak hydrolysis (generally, k_{cat} of 0.5 to 2 s^{-1}); −, no measurable hydrolysis reported (generally, k_{cat} of <0.5 s^{-1}).
[b] Symbols: +, reported inhibition; ±, variable inhibition among β-lactamase family members; −, no inhibition reported.

Plasmid-Encoded Enzymes: KPC and GES

Two characteristics separate the KPC (for "*Klebsiella pneumoniae c*arbapenemase") carbapenemases from the other functional group 2f enzymes. First, the KPC enzymes are found on transferable plasmids; second, their substrate hydrolysis spectrum includes the aminothiazoleoxime cephalosporins, such as cefotaxime. Although the KPC β-lactamases are predominantly found in *K. pneumoniae*, there have been reports of these enzymes in *Enterobacter* spp. and in *Salmonella* spp. (14, 74, 137).

The first member of the KPC family was discovered through the ICARE surveillance project in a *K. pneumoniae* clinical isolate from North Carolina in 1996 (245). This isolate was resistant to all β-lactams tested, but carbapenem MICs decreased in the presence of clavulanic acid. Carbapenemase activity, first detected with a bioassay, was associated with a large plasmid that encoded the KPC-1 β-lactamase.

The discovery of KPC-1 was quickly followed by several reports of a single-amino-acid variant, KPC-2, along the east coast of the United States (137, 138, 246). KPC-2 was first identified in 2003 as the result of a point mutation in KPC-1 and appeared in four isolates with imipenem MICs of 2 to 8 μg/ml from Baltimore, MD, from 1998 to 1999. The KPC-2-producing gene resided on a transferable plasmid, and it was noted that while all isolates exhibited reduced susceptibility to imipenem, none were technically resistant according to approved CLSI (formerly NCCLS) breakpoints (138). KPC-2 was then described in another Maryland site on a plasmid in *Salmonella enterica* (137). Genetic regions around this KPC-2 gene contained three open reading frames with sequence homology to transposases.

Reports of KPC-2 in the New York, NY, area began to appear in 2004, with KPC-expressing *K. pneumoniae* currently an alarming problem (13, 15, 17). This is especially disturbing because New York has had large outbreaks of ESBL-producing klebsiellae (135, 177) for which carbapenems were considered to be one of the few treatment options (156). When ribotyping was conducted on the KPC-2-producing strains, the majority of the isolates were clonal, even when the surveillance included multiple hospitals in the New York metropolitan area. Notably, several of these reports described inconsistencies in recognizing some of these strains as carbapenem resistant, because carbapenem MICs were less than the approved MIC breakpoints (13, 15, 16, 118).

Concurrent with the increasing reports of KPC-2, a single-amino-acid variant of KPC-2, KPC-3, was reported from a 2000 to 2001 *Klebsiella pneumoniae* outbreak in New York (233). KPC-3 has also been detected in *Enterobacter* spp. (14), where MICs for imipenem were also not consistently in the resistant range. Kinetic analysis of the KPC-3 enzyme revealed a profile similar to those of KPC-1 and KPC-2, with a slight increase in the hydrolysis of ceftazidime (4).

After the rapid expansion of the KPC class of carbapenemases along the east coast of the United States, worldwide reports began to appear. A report from France in 2005 documented KPC-2 in a *K. pneumoniae* strain from a patient who had been in New York for medical treatment (145). KPC carbapenemases have recently been detected in Scotland (KPC-4 [GenBank accession no. AY700571]), Colombia (217), Israel (147), and China (229). The first detection of KPC-2 on a plasmid in *P. aeruginosa* has been reported; this represents a disturbing development in the spread of these carbapenemases (216).

KPC enzymes have the conserved active-site motifs S-X-X-K, S-D-N, and K-T-G of the class A β-lactamases and have the closest amino acid identity (~45%) to the SME carbapenemases. In addition, these β-lactamases have the conserved C69 and C238 residues that form a disulfide bond described for the SME and NMC/IMI enzymes. The structure of the KPC-2 β-lactamase, compared to the SME-1 and NMC-A carbapenemases and the TEM-1 and SHV-1 noncarbapenemases, reveals characteristics conserved among the carbapenemases. KPC-2, along with the other carbapenemases, had a decrease in the size of the water pocket and had the catalytic serine in a more shallow position of the active-site cleft (92). The combination of subtle active-site adjustments in the class A car-

bapenemases is proposed to allow carbapenems access to the catalytic site, resulting in the altered specificity of these enzymes.

KPC carbapenemases hydrolyze β-lactams of all classes, with the most efficient hydrolysis observed for nitrocefin, cephalothin, cephaloridine, benzylpenicillin, ampicillin, and piperacillin (Tables 3 and 4). Imipenem and meropenem, as well as cefotaxime and aztreonam, were hydrolyzed 10-fold-less efficiently than the penicillins and early cephalosporins. Weak but measurable hydrolysis was observed for cefoxitin and ceftazidime, giving the KPC family a broad hydrolysis spectrum that includes most β-lactam antibiotics.

Of the functional group 2f carbapenemases, the KPC family has the greatest potential for spread due to its location on plasmids, especially since it is most frequently found in *K. pneumoniae*, an organism notorious for its ability to accumulate and transfer resistance determinants. In addition, the clonal spread seen in several epidemics points to difficulties with infection control for this organism (13, 15–17, 233). Most worrisome, treatment of infections caused by these organisms is extremely difficult because of their multidrug resistance, which results in high mortality rates (15).

The GES/IBC family of β-lactamases is an infrequently encountered family that was first described in 2000 with reports of IBC-1 (for "integron-borne cephalosporinase") from an *E. cloacae* isolate in Greece (53) and GES-1 (for "Guiana extended spectrum") in a *K. pneumoniae* isolate from French Guiana (168). These enzymes differ by only two amino acid substitutions and possess the class A active site-motifs with the cysteine residues at Ambler positions 69 and 238 that have been found in the KPC, SME, and NMC/IMI families. Their amino acid sequences show them to be distantly related to these carbapenemases, with identities of 36% to KPC-2, 35% to SME-1, and 31% to NMC-A (174).

The genes encoding the GES family of enzymes were located in integrons on plasmids. Because the enzymes had a broad hydrolysis spectrum that included penicillins and extended-spectrum cephalosporins, they were initially classified as extended-spectrum β-lactamases (53). Their hydrolysis spectrum was expanded in 2001 to include imipenem, with the report of GES-2 in a clinical isolate of *P. aeruginosa* (174). GES-2, from a multidrug-resistant *P. aeruginosa* isolate from South Africa, had a single amino acid substitution of glycine to asparagine at position 170. Imipenem hydrolysis by GES enzymes was slow, with hydrolytic rates of ≤ 0.004 s^{-1} and 0.004 s^{-1} for GES-1 and GES-2, respectively. However, the hydrolytic efficiency for imipenem was 100-fold higher for GES-2, due to a 100-fold decrease in the K_m value. The GES-4 β-lactamase differed from GES-2 by three amino acids, one of which was a serine at position 170. Carbapenem k_{cat} values for purified GES-4 were higher than those for GES-2, with imipenem hydrolyzed at a rate of 0.38 s^{-1} (219).

Nomenclature of the GES/IBC family has undergone several revisions (105). A consensus nomenclature has been reached whereby the IBC names have been converted to the GES nomenclature (83, 105). At least nine GES variants have been described, with GES-9 recently identified in a *P. aeruginosa* isolate from France (164). Of these closely related enzymes, GES-2, GES-4, GES-5, and GES-6 have substitutions of as-

paragine or serine at position 170, associated with imipenem hydrolysis (105, 218).

Although rare, GES enzymes have been identified worldwide, with reports from Greece, France, Portugal, South Africa, French Guiana, Brazil, Argentina, Korea, and Japan (25, 33, 42, 86, 90, 155, 164, 168, 173, 186, 218–220). These enzymes have been most frequently associated with single occurrences. However, *P. aeruginosa* strains expressing GES-2 have caused a small nosocomial outbreak in eight patients (173), and six patients in Korea had infections caused by GES-5-producing *K. pneumoniae* (86).

CLASS B METALLO-β-LACTAMASES

The metallo-β-lactamases have been thoroughly reviewed recently (221, 224), and so this section serves as a summary and epidemiological update. This class of β-lactamases is characterized by the ability to hydrolyze carbapenems and by its resistance to the commercially available β-lactamase inhibitors but susceptibility to inhibition by metal ion chelators. The substrate spectrum is quite broad; in addition to the carbapenems, most of these enzymes hydrolyze cephalosporins and penicillins but lack the ability to hydrolyze aztreonam. The mechanism of hydrolysis is dependent on interaction of the β-lactams with zinc ions in the active site of the enzyme, resulting in the distinctive trait of their inhibition by EDTA, a chelator of Zn^{2+} and other divalent cations.

The first metallo-β-lactamases detected and studied were chromosomal enzymes present in environmental and opportunistic pathogenic bacteria such as *Bacillus cereus* (98, 109), *Aeromonas* spp. (78), and *Stenotrophomonas maltophilia* (188). These chromosomal enzymes were usually found in bacteria that also expressed at least one serine β-lactamase, with both β-lactamases inducible after exposure to β-lactams. Fortunately, with the exception of *S. maltophilia*, these bacteria have not been frequently associated with serious nosocomial infections, as they are generally opportunistic pathogens, and the chromosomal metallo-β-lactamase genes are not easily transferred.

Early classification based on functional analyses of purified proteins indicated that these β-lactamases were distinctive from the groups of enzymes that had a serine-based hydrolytic mechanism (21). Major distinctive properties included the requirement of Zn^{2+} for the efficient hydrolysis of β-lactams and a lack of inhibition by clavulanic acid and tazobactam. A defining aspect of their substrate spectrum was the ability to hydrolyze carbapenems (Table 4). Interestingly, not all of the metallo-β-lactamases readily hydrolyzed nitrocefin, the popular colorimetric indicator of β-lactamase activity (130). The "hidden" carbapenemases of *Aeromonas* spp. hydrolyzed nitrocefin and other cephalosporins imperceptibly and could be detected only by using a carbapenem bioassay or hydrolysis test (64, 65, 196, 223, 241). Recently, the VIM-2 enzyme was shown to share this property and was detected on IEF gels by an imipenem hydrolysis detection method (171).

Since the initial identification of the metallo-β-lactamases, a considerable amount of sequencing work has demonstrated high variability in primary sequences and in molecular structures. The first metallo-β-lactamases for which an amino acid sequence was determined was the BCII metallo-β-lactamase

from *Bacillus cereus* (77), the prototypical metallo-β-lactamase for many years. Extensive molecular characterization, including biochemical and crystallization studies, has also been completed with the CcrA (CfiA) chromosomal metallo-β-lactamase that appears in a small percentage of *Bacteroides fragilis* strains (32, 242). Amino acid sequence identities are as low as 23% across the metallo-β-lactamases, including enzymes such as CcrA, CphA, and L1 (Table 1) (182, 224). However, all of the enzymes have conserved residues that bind zinc at two sites (see "Classification Schemes," above, and Table 1) and exhibit a well-conserved active site, as demonstrated through sophisticated modeling analyses (127) and in the crystal structures published to date (31, 32, 34, 44, 52, 141, 214).

In contrast to the chromosomal metallo-β-lactamases, whose presence is directly correlated with the prevalence of the producing species, there has been a dramatic increase in the detection and spread of the acquired or transferable families of these metalloenzymes. The most common metallo-β-lactamase families include the VIM, IMP, GIM, and SIM enzymes, which are located within a variety of integron structures, where they have been incorporated as gene cassettes. When these integrons become associated with plasmids or transposons, transfer between bacteria is readily facilitated.

Transferable imipenem resistance was first detected in Japan, initially in a *P. aeruginosa* isolate, in 1990 (228), followed by a second report of a transferable carbapenemase in *B. fragilis* (11). The transferable *B. fragilis* enzyme was one of the first characterized metallo-β-lactamases, but this species has not caused widespread clinical outbreaks in Japan. In contrast, IMP-1 (for "active on imipenem"), located on a conjugative plasmid in the *P. aeruginosa* clinical isolate (228), was found on an integron in *S. marcescens* and other *Enterobacteriaceae* in Japan (7, 72, 80, 195). This enzyme hydrolyzed imipenem, penicillins, and extended-spectrum cephalosporins but not aztreonam. The hydrolytic activity was inhibited by EDTA and restored by the addition of Zn^{2+}. The first member of the IMP family found in Europe was in an *A. baumannii* isolate from Italy, which produced a related enzyme, IMP-2, as the first cassette on a class 1 integron (184). Since that time, the IMP family has been found throughout the world, with the most recent spread to the United States and Australia (62, 159). The historical discovery of IMP-type β-lactamases is well documented in the recent review by Walsh et al. (224). Currently, the IMP family members number up to 18 in the published literature, with 23 assigned IMP sequences listed on the β-lactamase nomenclature website (http://www.lahey.org/Studies/ [updated 17 November 2006]).

Another prevalent family of integron-associated metallo-β-lactamases is composed of the VIM enzymes. VIM-1 (for "Verona integron-encoded metallo-β-lactamase") was first isolated in Verona, Italy, in 1997 (101), with the identification of VIM-2 in France in 1996 subsequently reported (171). Both of these enzymes were initially found in *P. aeruginosa* clinical isolates and resided in class 1 integrons. The VIM family currently consists of 14 members (http://www.lahey.org/Studies/ [updated 17 November 2006]), with occurrences mostly in *P. aeruginosa* within multiple-integron cassette structures. VIM-2 has the dubious distinction of being the most-reported metallo-β-lactamase worldwide (224).

Identification of the SPM-1 metallo-β-lactamase defined a

new family with 35.5% amino acid identity to IMP-1 (206). SPM-1 (for "Sao Paulo metallo-β-lactamase") was first isolated in a *P. aeruginosa* strain in Sao Paolo, Brazil. Since the initial report, single clones of SPM-1-containing *P. aeruginosa* have caused multiple hospital outbreaks with high mortality in Brazil (126, 169, 251). Genetic analysis of regions around the SPM-1 gene revealed that it was not part of an integron but instead was associated with common regions that contain a new type of transposable structure with potential recombinase and promoter sequences (169, 206).

GIM-1 (for "German imipenemase") was isolated in Germany in 2002 (26). GIM had approximately 30% homology to VIM, 43% homology to IMPs, and 29% homology to SPM. GIM-1 has characteristics similar to those of the other acquired metallo-β-lactamases in that it was found in five clonal *P. aeruginosa* isolates within a class 1 integron on a plasmid. At this time, it has not been reported elsewhere in the world.

The latest family of acquired metallo-β-lactamases to be described comes from Korea. The enzyme SIM-1 (for "Seoul imipenemase") has the closest amino acid identity to the IMP family (64 to 69%). SIM-1 was discovered in a large-scale screen of 1,234 imipenem-resistant *Pseudomonas* sp. and *Acinetobacter* sp. isolates, of which 211 (17%) were positive for metallo-β-lactamases. In this screening study, mostly VIM (74%) and IMP (22%) alleles were identified; however, seven putative metallo-β-lactamase-producing *A. baumannii* isolates were negative by PCR testing. Investigation with PCR primers designed to amplify entire integrons revealed that they had the novel SIM-1 metallo-β-lactamase located within a class 1 integron (104). Two clonal groups, of four and three strains, carried the same integron, suggesting independent acquisition events.

Since their initial discoveries, SPM, GIM, and SIM metallo-β-lactamases have not spread beyond their countries of origin. However, VIM and IMP continue to be detected worldwide, with an overall trend of these two metallo-β-lactamases moving beyond *P. aeruginosa* and into the *Enterobacteriaceae* (Table 5).

Recent Epidemiology

Overall, worldwide susceptibility to carbapenems is 98% among the *Enterobacteriaceae*, whereas imipenem susceptibility ranges from 60% to 83% for *P. aeruginosa* and *A. baumannii* (2004 to 2005 surveys) (93, 209, 210). The current epidemiology of metallo-β-lactamase production generally follows patterns of increasing occurrences that are country specific. Presumably this is due to multiple factors, including antibiotic usage, dosing regimens, and local hospital practices concerning isolation of patients with multiresistant pathogens. While outside the focus of this review, it should be noted that the loss of the OprD porin in *P. aeruginosa*, not the acquisition of carbapenemases, is the most common mechanism associated with imipenem resistance in this pathogen (113, 176).

In a recent survey from Korea of 15,960 gram-negative clinical isolates (247), metallo-β-lactamases were found in 36 of 581 imipenem-resistant isolates. In *P. aeruginosa*, VIM-2-like enzymes were the majority of metallo-β-lactamases in this sample; however, two strains tested positive by PCR for IMP-1-like enzymes. For the *Acinetobacter* species, 26.5% (136 of

TABLE 5. Worldwide emergence of metallo-β-lactamases in the *Enterobacteriaceae*

Country	Isolation date[a]	Publication date	Organism	Enzyme[b]	No. of isolates	Location	Plasmid type	Integron type	Reference(s)
Australia	2002	2004	*K. pneumoniae*	IMP-4	1	Plasmid	Not tested	1	172
Australia			*E. coli*	IMP-4	1	Plasmid	Not tested	1	172
Australia	NA	2005	*K. pneumoniae*	IMP-4	2	Plasmid	Conjugative	1	43
			E. cloacae	IMP-4	1	Plasmid	Conjugative	1	43
			C. amalonaticus	IMP-4	1	Plasmid	Conjugative	1	43
Brazil	2003	2005	*K. pneumoniae*	IMP-1	1	Chromosome		1	110
China	NA	2001	*C. youngae*	IMP-4	1	Plasmid	Conjugative	?	63
Greece	2001	2003	*E. coli*	VIM-1	1	Plasmid	Conjugative	1	136
Greece	2001	2004	*E. coli*	VIM-1	4 (clonal)	Plasmid	Conjugative	1	193
Greece	2002	2003	*K. pneumoniae*	VIM-1	17	Plasmid	Conjugative	1	54
Greece	2003	2005	*E. cloacae*	VIM-1	1	Chromosome		1	48
Greece	2003–2005	2006	*K. pneumoniae*	VIM-1	5 (clonal)	Plasmid	Conjugative	1	117
Greece	2004–2005	2005	*K. pneumoniae*	VIM-1	27 (clonal)	Plasmid	Conjugative	1	79
France	2003–2004	2006	*K. pneumoniae*	VIM-1	8 (clonal)	Plasmid	Nonconjugative	1	91
Italy	2002	2004	*K. pneumoniae*	VIM-4	1	Plasmid	Conjugative	Not tested	120
			E. cloacae	VIM-4	1	Plasmid	Conjugative	Not tested	120
Japan	1993	1995	*S. marcescens*	IMP-1-like	4	Multiple plasmids	Conjugative and nonconjugative	Not tested	80
Japan	1994–1995	1996	*K. pneumoniae*	IMP-1-like	1	Not tested	Not tested	3	195
			S. marcescens	IMP-1-like	9	Not tested	Not tested	3	195
Japan	NA	1998	*S. flexneri*	IMP-3	1	Plasmid	Conjugative	1	81, 152
Japan	1991–1996	1998	*S. marcescens*	IMP-1-like	13 (some clonal)	Not tested		Not tested	72
			C. freundii	IMP-1-like	1	Not tested		Not tested	72
Japan	1996	2001	*S. marcescens*	IMP-6	1	Plasmid	Conjugative	1	244
Japan	1998–2000	2003	*S. marcescens*	IMP-1	5	Not tested		Not tested	235
			C. freundii	IMP-1	1	Not tested		Not tested	8
			P. vulgaris	IMP-1	1	Not tested		Not tested	8
Japan	2001–2002	2003	*S. marcescens*	IMP-1-like	47	Not tested		1	197
			K. pneumoniae	IMP-1-like	23	Not tested		1	197
			E. coli	IMP-1-like	17	Not tested		1	197
			E. cloacae	IMP-1-like	5	Not tested		1	197
			C. freundii	IMP-1-like	3	Not tested		1	197
			K. oxytoca	IMP-1-like	2	Not tested		1	197
			P. rettgeri	IMP-1-like	2	Not tested		1	197
			M. morganii	IMP-1-like	1	Not tested		1	197
			E. aerogenes	IMP-1-like	1	Not tested		1	197
Portugal	NA	2005	*K. oxytoca*	VIM-2	4 (clonal)	Plasmid	Nonconjugative	1	30
Singapore	1996	1999	*K. pneumoniae*	IMP-1	1	Plasmid	Conjugative	Not tested	95
South Korea	2000	2003	*E. cloacae*	VIM-2	1	Chromosome		1	87
South Korea	2000	2002	*S. marcescens*	VIM-2	1	Not tested		1	250
South Korea	2003–2004	2006	*K. pneumoniae*	VIM-2-like	2	Not tested		Not tested	250
			E. cloacae	VIM-2-like	1	Not tested		Not tested	250
			S. marcescens	VIM-2-like	1	Not tested		Not tested	250
Spain	2003	2005	*K. pneumoniae*	VIM-1	1	Plasmid	Conjugative	1	207
			E. coli	VIM-1	1	Plasmid	Conjugative	1	207
Taiwan	1998	2001	*K. pneumoniae*	IMP-8	1	Plasmid	Conjugative	1	238
Taiwan	1999–2000	2002	*E. cloacae*	IMP-8	36 (mostly clonal)	Plasmid	Conjugative	Not tested	237
			C. freundii	VIM-2	1	Plasmid	Conjugative	Not tested	237
Tunisia	2005	2006	*K. pneumoniae*	VIM-4	20 (clonal)	Plasmid		1	97
Turkey	Before 2002	2005	*E. cloacae*	VIM-5	1	Plasmid	Nonconjugative	1	47

[a] NA, not available.
[b] IMP-1-like and VIM-2-like enzymes were detected by PCR or colony hybridization but were not sequenced.

513) of the imipenem-resistant strains carried metallo-β-lactamases: 64% were VIM-2-like, 29% were IMP-1-like, and 7% were SIM-like. Among the *Enterobacteriaceae*, resistance to imipenem was 2% for *K. pneumoniae* and <1% for *E. cloacae* and *S. marcescens*, which tested positive by PCR for VIM-2.

IMP metallo-β-lactamases continue to persist as the major metalloenzymes found in Japan, both at a local (148) and a country-wide (94) level. However, VIM-2-like genes have also been detected in *P. aeruginosa*. IMP-1 and IMP-2-like enzymes were found in *Acinetobacter* spp., as well as *S. marcescens*, *P. rettgeri*, *C. freundii*, *E. cloacae*, and *M. morganii*. Among almost 20,000 clinical gram-negative isolates from 13 clinical laboratories, the overall rate of metallo-β-lactamase detection was 0.5% in Japan. In these reports, there was as much as 2.6% metallo-β-lactamase detection in imipenem-resistant *P. aeruginosa*; *S. marcescens* had the highest rate of IMP gene detection in Japan, at 3%. In a SENTRY surveillance study conducted in Japan, 1.1% of the *P. aeruginosa* strains expressed metallo-β-

lactamases (88); among these, IMP alleles were the only metallo-β-lactamases detected.

The first metallo-β-lactamase reported from China was IMP-4, which was found on a plasmid in *C. youngae* and reported in a 2001 publication (63), followed by a report of IMP-1 in a *P. aeruginosa* isolate (226). Recently, IMP-9 has emerged in this country, as reported in a surveillance study that included 11 hospitals (234). The IMP-9 genes were all on identical integrons, and only two strains that came from the same ward were related, based on random amplified polymorphic DNA typing. A very large plasmid (~450 kb) was shown to carry the IMP-9 gene by conjugation experiments, suggesting that the spread of this IMP-containing integron was through horizontal transfer.

The VIM-2 metallo-β-lactamase was first reported in China only in 2006 (227). This single *P. aeruginosa* isolate occurred in the same hospital as the IMP-1 isolate (226). At this point, the route for emergence of these enzymes is unknown, but further

epidemiological analysis, sequencing of the integrons, and typing of the strains could answer questions about whether the strains imported or acquired metallo-β-lactamases independently.

In Taiwan, IMP-1 was reported in two *A. baumannii* isolates that were unrelated by PFGE. In these isolates, the IMP-1 gene was on two different plasmids within class 1 integrons (111). VIM-2 and VIM-3 have also been detected within integrons in multidrug-resistant *P. aeruginosa* (236).

In southern Europe, particularly in Italy, there has been almost continuous detection of VIM and IMP metallo-β-lactamases throughout many countries over the past decade. In Italy, a recent SENTRY study for the years 2001 and 2002 found that 25 of 383 (6.5%) *P. aeruginosa* isolates from three medical centers carried metallo-β-lactamases (88). The majority of metallo-β-lactamases identified were VIM-1, but IMP-13 was also detected. Some of the VIM-1-expressing isolates from Rome and Catania carried the integron described in the original VIM-1 from Verona; differences in ribotypes demonstrated the mobility of this integron. Several unique integron cassette arrangements were reported, including some strains possessing as many as four integrons, thus leaving open the possibility for more integron gene rearrangements (100). In another study reported by Pagani et al., IMP-13 was responsible for a large clonal outbreak of at least 86 metallo-β-lactamase-producing *P. aeruginosa* strains in southern Italy (154). The integron, which was the same as the IMP-13 found in the SENTRY study, was localized to the chromosome and not transferable to *P. aeruginosa* or *Escherichia coli* by conjugation. Another surveillance study of 506 *P. aeruginosa* isolates from a single center in Varese showed 82% IPM susceptibility, but with only four strains carrying metallo-β-lactamases, (VIM-1 and VIM-2), demonstrating the regional variation in resistance patterns due to these enzymes (121).

More frequent reports of metallo-β-lactamases are now being published from other European countries, documenting further detections of acquired carbapenem resistance. *P. aeruginosa* isolates with integron-associated VIM alleles have been reported in Germany (66), Turkey (10), Croatia (190), Hungary (107, 108), and Poland (45).

Metallo-β-lactamase outbreaks in South America are dominated by the SPM and VIM families. In a recent SENTRY study of 183 *P. aeruginosa* isolates, 44.8% were imipenem resistant; of these, 36 isolates were positive for metallo-β-lactamases. The majority of the isolates were positive for SPM-1-like genes by PCR, (55.6%), followed by VIM-2-type (30.6%) and IMP-1-like genes in three isolates (8.3%) (88, 187). While SPM-1 has been confined to Brazil, other countries in South America that have reported metallo-β-lactamases are Argentina, Chile, and Venezuela (88), as well as Colombia, where both VIM-2 and VIM-8 were found in *P. aeruginosa* (36, 217).

Emergence of metallo-β-lactamase-mediated carbapenem resistance has spread to the United States and Canada, with reports of both VIM and IMP metallo-β-lactamases in *P. aeruginosa*. VIM-7 was isolated in Texas in 2001 from a single strain that carried the integron on a conjugative plasmid (204). More recently, VIM-2 has also been detected in Texas (1). VIM-2 was also found in Chicago, IL, in an outbreak setting of clonal *P. aeruginosa* involving 17 patients admitted from 2002 to 2004 (115), where the metallo-β-lactamase was present on

an integron. Metallo-β-lactamases have also emerged as outbreaks of VIM-2- and IMP-7-producing *P. aeruginosa* in Canada (55, 163) and in a single *P. aeruginosa* isolate from the southwestern United States that produced IMP-18 (62).

Australia has also reported detection of metallo-β-lactamases. The first metalloenzyme that emerged on the Australian continent was IMP-4, reported in 2004 in a single *P. aeruginosa* clinical isolate (159) and in single *K. pneumoniae* and *E. coli* isolates (172), followed by an outbreak of IMP-4-producing gram-negative pathogens (158). Recently, an *Aeromonas junii* isolate from Australia was found to have two carbapenemases: a class D OXA-58 and an IMP-4 metallo-β-lactamase (160).

Other areas of the world that have recently reported metallo-β-lactamases include the Middle East, where VIM-2 was the first reported metallo-β-lactamase. It was detected by PCR in an imipenem-resistant *P. aeruginosa* isolate from Saudi Arabia (58). Yong et al. recently reported the detection of metallo-β-lactamases from India by using an EDTA disk test (248), where 13 of 200 (7.5%) *Pseudomonas* sp. and *Acinetobacter* sp. isolates were positive for the production of metallo-β-lactamases. Although further analysis of these enzymes is not yet available, awareness and early detection may prevent uncontrolled outbreaks due to these pathogens (59).

CLASS D SERINE-CARBAPENEMASES: THE OXA β-LACTAMASES

OXA (for "oxacillin-hydrolyzing") β-lactamases represented one of the most prevalent plasmid-encoded β-lactamase families in the late 1970s and early 1980s (129, 132, 198). When the molecular class D OXA β-lactamases were placed in a separate molecular class from the other serine β-lactamases (76), they had been identified mainly in the *Enterobacteriaceae* and *P. aeruginosa* (23, 144) and were functionally described as penicillinases capable of hydrolyzing oxacillin and cloxacillin (21). They were in general poorly inhibited by clavulanic acid and EDTA and known to have a large amount of variability in amino acid sequences (22). OXA-11, the first extended-spectrum variant of OXA-10 (previously known as PSE-2), was described in 1993 (61). The extended-spectrum variants OXA-11, OXA-15, OXA-18, and OXA-45 had hydrolysis rates for ceftazidime that varied from 1% to 1,150% relative to the hydrolysis rate of penicillin, but imipenem hydrolysis was not detected (38, 61, 162, 205). Currently there have been 102 unique OXA sequences identified (http://www.lahey.org /Studies/), of which 9 are extended spectrum β-lactamases and at least 37 are considered to be carbapenemases (Table 6) (225).

The first OXA β-lactamase with carbapenemase activity was described by Paton et al. in 1993. The enzyme was purified from a multidrug-resistant *A. baumannii* strain that was isolated in 1985 from a patient in Edinburgh, Scotland (157). Biochemical characterization revealed a β-lactamase with a pI value of 6.65 that was poorly inhibited by clavulanic acid and EDTA (Table 4). Imipenem hydrolysis could not be measured spectrophotometrically but was readily detected with a microbiological assay plate. The enzyme was designated ARI-1 (for "*Acinetobacter* resistant to imipenem") and was later demonstrated to reside on a large plasmid (191). Sequencing of the ARI-1 enzyme revealed that it belonged to the OXA class D

TABLE 6. Carbapenemase subgroups of the OXA family
of β-lactamases

Cluster	Enzyme subfamily	Additional OXA member(s)	Reference
1	OXA-23 (ARI-1)	OXA-27, OXA-49	225
2	OXA-24	OXA-25, OXA-26, OXA-40, OXA-72	225
3	OXA-51	OXA-64 to OXA-71, OXA-75 to OXA-78, OXA-83, OXA-84, OXA-86 to OXA-89, OXA-91, OXA-92, OXA-94, OXA-95	213, 225
4	OXA-58	None	225
5	OXA-55	OXA-SHE	225
6	OXA-48	OXA-54, OXA-SAR2	225
7	OXA-50	OXA-50a to OXA-50d, PoxB	225
8	OXA-60	OXA-60a to OXA-60d	225
9	OXA-62	None	192

family of β-lactamases, and the enzyme was later renamed OXA-23 (41). OXA-23 represented a new subset of the OXA family, with the highest amino acid identity to OXA-5 and OXA-10 at 36%. A recent review of the OXA β-lactamases includes many additional details of these enzymes (225).

The vast majority of OXA carbapenemases have been discovered in the opportunistic gram-negative pathogen *Acinetobacter baumannii*. By 1998, carbapenem-hydrolyzing β-lactamases had been identified in *Acinetobacter* species clinical isolates throughout the world (2). For example, OXA-23 has been identified in outbreaks of carbapenem-resistant *Acinetobacter* in Brazil, the United Kingdom, Korea, and Tahiti (37, 85, 142, 211). OXA-24 and OXA-40, which differ by two amino acids, were found in clonal *Acinetobacter* outbreaks in hospitals from Spain and Portugal (12, 40, 119). OXA-40 was also the first carbapenem-hydrolyzing oxacillinase reported in the United States (116). *Acinetobacter* strains with OXA-23 and OXA-58 carbapenemases caused multiple infections in military and civilian personnel serving in Iraq and Afghanistan from 2003 to 2005 (75).

At the latest count, there are nine major subgroups of OXA carbapenemases, based on amino acid homologies, as summarized in Table 6 (18, 167, 225). Subgroups 1, 2, and 3 are based on the sequences of OXA-23, OXA-24, and OXA-51, respectively (213). OXA-51-like enzymes have been found in all *A. baumannii* strains tested and may be a natural component of the chromosome in a subpopulation of that species (69). OXA-58, less that 50% identical to other members of the OXA family, stands alone in subgroup 4 and has been found in *Acinetobacter* spp. from France, Greece, Italy, Romania, Turkey, Argentina, and Kuwait (28, 68, 125, 170, 215). OXA-55 and OXA-SHE, both from *Shewanella algae*, form the fifth group (71). The OXA-48 enzyme forms the sixth subgroup, along with OXA-54 and additional oxacillinases found in the environmental bacteria *Shewanella* spp. (165–167). In contrast to the sharp increase in worldwide reports of OXA-expressing *Acinetobacter* strains, OXA-48 was discovered in a clinical *K. pneumoniae* isolate from Turkey (167). This OXA variant was plasmid encoded and had less than 50% amino acid identity to the other OXA members. This enzyme also had the highest reported imipenem k_{cat} value, 2 s^{-1}, which represents the

highest hydrolysis rate of all of the published kinetic parameters for the OXA enzymes.

The OXA-50-like enzymes in *P. aeruginosa* form the seventh group and include a set of enzymes that have been referred to as the *poxB* enzymes. These PoxB oxacillinases have been reported to be commonly present on the chromosomes of many strains of *P. aeruginosa* (40 of 70 strains tested) (96) and are thought to be part of the natural component of β-lactamases in that species, but they may not be expressed in all strains and do not cause carbapenem resistance (imipenem MIC, ≤1 μg/ml) (56, 96). Species-specific OXA enzymes include the subgroup 8 OXA-60 family, considered to be a natural component of the genome of *Ralstonia pickettii* (57), and the subgroup 9 OXA-62, identified as a species-specific oxacillinase in *Pandoraea pnomenusa* (192).

The OXA β-lactamases display a wide variety of amino acid sequences. Among those with carbapenem-hydrolyzing activity, there is 40% to 70% amino acid identity between groups. Within a group the identity is greater than or equal to 92.5% (225). If OXA β-lactamases without carbapenem-hydrolyzing activity are considered, amino acid identities can be as low as 18%, for example, when OXA-1 is compared with OXA-58 (170).

The molecular structures of the OXA β-lactamases have been analyzed with the DBL numbering system (35), a classification based on the similarity of motifs around the active sites of the class A enzymes (6). The catalytic serine residue lies in the S-T-F-K tetrad at positions 70 to 73 (102), where the serine and lysine are conserved in both class A and class D enzymes, as well as in penicillin-binding proteins. The Y-G-N motif at positions 144 to 146 and the K-T-G at DBL 216 to 218 are highly conserved among the serine-based β-lactamases. The carbapenem-hydrolyzing OXA subgroups 1 and 2 share a substitution of F for Y in the Y-G-N motif, but this is not necessary for imipenem hydrolysis, as the subgroup 3 enzymes and OXA-58 retain the Y-G-N at this position.

The first crystal structure of the class D carbapenemases to appear in the literature was that of OXA-24, which was compared to the noncarbapenemase OXA-10 (189). Two amino acids, Tyr-112 and Met-223, make access to the active site smaller and more hydrophobic, allowing favorable interactions with carbapenems. These residues are found in other subgroups of OXA carbapenemases, suggesting that the altered active-site access is an important contributing factor to the carbapenem-hydrolyzing activity among these enzymes.

The catalytic mechanism of the OXA β-lactamases shares features with other serine carbapenemases. Substrate and enzyme form a covalent acyl intermediate at the catalytic serine, which is subsequently deacylated to yield the inactivated antibiotic hydrolyzed at the C-N bond of the β-lactam ring. In addition, CO_2 may influence the kinetics of some OXA enzymes, due to carboxylation at lysine 70 (Lys 73 in the DBL scheme) in these class D enzymes. This carbamate activates the catalytic serine side chain so that the acyl intermediate can form (131, 153). To ensure that this modification occurs in studies with isolated enzymes, some researchers add 10 mM $NaHCO_3$ to their reactions (57, 69, 192).

OXA enzymes are difficult to purify due to low yield (3) and difficult to characterize biochemically due to low hydrolysis

rates and biphasic kinetics for some substrates. The OXA carbapenemases that have been characterized biochemically have measurable hydrolytic activity against the penicillins, some cephalosporins, and imipenem (225). In general, imipenem hydrolysis, which is faster than meropenem hydrolysis, is slow, with the highest k_{cat} values for the group 6 OXA-54 and OXA-48 enzymes at 1 s^{-1} and 2 s^{-1}, respectively. The K_m values for imipenem are also low, ranging from 2 to 20 μM, indicating that the OXA enzymes have very high affinity for these substrates. In contrast, extended-spectrum cephalosporins are not measurably hydrolyzed by the OXA carbapenemases or are hydrolyzed very poorly (225).

The hydrolysis of carbapenems by the class D oxacillinase family is weak, but Heritier et al. (70) demonstrated that plasmid-encoded OXA-23 and OXA-58 enzymes contributed to carbapenem resistance in *A. baumannii* after transformation of the OXA plasmids into carbapenem-susceptible *A. baumannii* strains. In addition, when a chromosomally located OXA-40 gene was inactivated, susceptibility to carbapenems was observed. Efflux by an overexpressed AdeABC pump was also shown to contribute to carbapenem resistance.

DETECTION OF CARBAPENEMASES

MICs

Detection of carbapenemase activity in a clinical isolate can be challenging for a clinical microbiology laboratory. The first cause for suspicion that a carbapenemase is involved in a clinical infection is an elevated carbapenem MIC. Among *P. aeruginosa* strains with VIM, IMP, GIM, SIM, and SPM metallo-β-lactamases, imipenem MICs have been reported in the range of 8 to >128 μg/ml (26, 101, 104, 126, 171, 204, 228). However, when the genes for these enzymes were transferred into *E. coli*, the observed imipenem MIC was usually much lower, sometimes as low as 0.5 μg/ml. This demonstrates the presence of other mechanisms contributing to the carbapenem resistance in *P. aeruginosa*, such as intrinsic impermeability, possibly coupled with an efflux mechanism. This effect of low-level transferable resistance has also been observed in *K. pneumoniae* and *A. baumannii* with metallo-β-lactamases (39, 184), as well as with OXA carbapenemases (12, 170, 192). Many OXA carbapenemases have been found in *A. baumannii*, where MICs for imipenem are usually higher than 8 μg/ml, but *E. coli* cells expressing these enzymes have imipenem MICs of ≤2 μg/ml (225).

Elevated carbapenem MICs are generally predictive of carbapenemase production in the *Enterobacteriaceae*, but full clinical resistance is not always seen. In a set of 19 *K. pneumoniae* isolates with imipenem MICs in the susceptible range of 1 to 4 μg/ml, a metallo-β-lactamase was suggested on the basis of disk testing with imipenem in the presence and absence of EDTA and was then confirmed by PCR as VIM-1 (161). A collection of five related *K. pneumoniae* strains with the VIM-1 gene demonstrated imipenem MICs ranging from 2 to 64 μg/ml (susceptible to high-level resistance) (117). Decreased permeability due to the absence of the outer membrane porin OmpK36 was a contributing factor in the most resistant isolate, with an imipenem MIC of 64 μg/ml.

The KPC serine carbapenemases also have been reported to be difficult to detect (14, 16, 138). They are often associated with imipenem MICs as low as 2 μg/ml (138), and a low inoculum has resulted in susceptible MICs by broth microdilution (15). To document inconsistencies in the detection of KPC-producing *K. pneumoniae* according to the testing method, Tenover et al. tested 15 characterized imipenem- and meropenem-nonsusceptible KPC-producing isolates for imipenem and meropenem resistance using CLSI broth microdilution, Etest, MicroScan WalkAway, BD Phoenix Sensititre Autoreader, VITEK, and VITEK2. The automated systems reported carbapenem susceptibility in this collection of isolates ranging from 6.7% to 87%, depending on the system used (203). Day-to-day variation was also noted. In addition to the variable results with the automated systems, Etest results were inconsistent due to colonies present in the zones of inhibition.

The failure of automated systems to consistently detect KPC-producing isolates indicates the need for improved methodology. One possibility is to screen isolates for resistance to ertapenem, which had the highest sensitivity for detecting KPC-expressing isolates (16). However, the specificity may be reduced due to resistance from other mechanisms, such as AmpC or ESBL production coupled with porin loss (N. Woodford, J. Dallow, R. Hill, M. F. Ralepou, R. Pike, M. Ward, M. Warner, and D. Livermore, presented at the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 27 to 30 September 2006).

The NMC and IMI serine carbapenemases in *E. cloacae* are associated with imipenem MICs of 16 to 32 μg/ml, with ceftazidime MICs often 2 μg/ml (149, 183). In *S. marcescens*, the SME family of β-lactamases should be suspected if an isolate displays this pattern of high-level imipenem resistance associated with ceftazidime susceptibility.

Microbiological Tests with Inhibitors

The disk approximation test with EDTA or 2-mercaptoproionic acid is often used as a screen for metallo-β-lactamase producers (8, 248). In this test, the zone of inhibition around a β-lactam disk is altered by the action of the inhibitor on the metallo-β-lactamase in the test organism. Imipenem, ceftazidime, and cefepime have been used for this test. The sensitivity with the imipenem-EDTA disk method was 100% for *Pseudomonas* spp. and 95.7% for *Acinetobacter* spp. (248). In one study that compared different combinations of antibiotics and inhibitors, imipenem-EDTA combinations were the most sensitive for the detection of metallo-β-lactamase-producing *Pseudomonas* and *A. baumannii*, while ceftazidime-clavulanate with EDTA was the most accurate for *K. pneumoniae* and cefepime-clavulanate with EDTA was the most accurate for *E. cloacae* and *C. freundii*, with an overall sensitivity for this method of 86.7% (239).

Etest strips for metallo-β-lactamase testing are also available as imipenem and imipenem-EDTA combinations (AB BIODISK, Solna, Sweden). A positive test for a metallo-β-lactamase is interpreted as a threefold-or-greater decrease in the imipenem MIC in the presence of EDTA. This test strip produced a sensitivity of 94% and a specificity of 95% when examined with a set of 138 characterized metallo-β-lactamase producers (222). However, false-negative results have been reported for the Etest when an isolate had an imipenem MIC

TABLE 7. PCR primers for the detection of β-lactamases[a]

Enzyme family	Primer[b]	Primer sequence (5′–3′)	GenBank accession no.	Nucleotide positions (in GenBank)	Fragment size (bp)	Entire coding region[c]	Reference
Class A carbapenemases							
NMC	NMC1*	GCATTGATATACCTTTAGCAGAGA	Z21956	68–191	2,158	Yes	181
	NMC4	CGGTGATAAAATCACACTGAGCATA		2225–2201			
SME	IRS-5	AGATAGTAAATTTTATAG	Z28968	5–22	1,138	Yes	179
	IRS-6	CTCTAACGCTAATAG		1142–1128			
IMI	IMI-A	ATAGCCATCCTTGTTTAGCTC	U50278	1291–1310		No	9
	IMI-B	TCTGCGATTACTTTATCCTC		2108–2089	818		
KPC	KPC forward	ATGTCACTGTATCGCCGTCT	AF297554	131–150	893	Yes*	13
	KPC reverse	TTTTCAGAGCCTTACTGCCC		1023–1004			
GES	GES-C	GTTTTGCAATGTGCTCAACG	AF326355	176–195	371	No	230
	GES-D	TGCCATAGCAATAGGCGTAG		527–546			
Class D oxacillinases							
Subgroup 1 (OXA-23)	P5	AAGCATGATGAGCGCAAAG	AJ132105	785–803	1,066	Yes	41
	P6	AAAAGGCCCATTTATCTCAAA		1850–1830			
Subgroup 2 (OXA-24)	Forward	GTACTAATCAAAGTTGTGAA					3
	Reverse	TTCCCCTAACATGAATTTGT		1023		Yes	
Subgroup 3 (OXA-69)	OXA-69A	CTAATAATTGATCTACTCAAG	AY859527	Primers external to GenBank sequence	975	Yes	69
	OXA-69B	CCAGTGGATGGATGGATAGATTATC					
Subgroup 4 (OXA-58)	Pre-OXA-58prom+	TTATCAAAATCCAATCGGC	AY570763	72–90	934	Yes	68
	PreOXA-58B	TAACCTCAAACTTCTAATTC		1005–986			
Subgroup 5 (*Shewanella* OXA-55)	OXA-55/1	CATCTACCTTTAAAATTCCC	AY343493	Primers external to GenBank sequence		Yes	71
	OXA-55/2	AGCTGTTCCTGCTTGAGCAC		969–917			
Subgroup 6 (OXA-48)	OXA-48A	TTGGTGGCATCGATTATCGG	AY236073	2218–2237	744	No	167
	OXA-48B	GAGCACTTCTTTTGTGATGGC		2961–2941			
Subgroup 7 (OXA-50)	S	AATCCGGCGCTCATCCATC	AE004091		869	Yes	56
	AS	GGTCGGCGACTGAGGCGG					
Subgroup 8 (OXA-60)	OXA-60 A	AAAGGAGTTGTCTCATGCTGTCTCG	AF525303	2757–2782			57
	OXA-60 B	AACCTACAGGCGCGCGTCTCAC GGTG		3605–3579		Yes	
Multiplex PCR for OXAs in *A. baumannii*	OXA-51-like	TAATGCTTTGATCGGCCTTG			353		232
		TGGATTGCACTTCATCTTGG					
	OXA-23-like	GATCGGATTGGAGAACCAGA			501		
		ATTTCTGACCGCATTTCCAT					
	OXA-24-like	GGTTAGTTGGCCCCCTTAAA			246		
		AGTTGAGCGAAAAGGGGATT					
	OXA-58-like	AAGTATTGGGGCTTGTGCTG			599		
		CCCCTCTGCGCTCTACATAC					
Class B metalloenzymes							
IMP-1	Forward	TGAGCAAGTTATCTGTATTC			740	Yes*	240
	Reverse	TTAGTTGCTTGGTTTTGATG					
IMP-2	Forward	GGCAGTCGCCCTAAAACAAA			737	Yes	240
	Reverse	TAGTTACTTGGCTGTGATGG					
VIM-1	Forward	TTATGGAGCAGCAACCGATGT			920	Yes	240
	Reverse	CAAAAGTCCCGCTCCAACGA					
VIM-2	Forward	AAAGTTATGCCGCACTCACC			865	Yes	240
	Reverse	TGCAACTTCATGTTATGCCG					
SPM-1	SPM-1F	CCTACAATCTAACGGCGACC	AJ492820	514–533	650	No	26
	SPM-1R	TCGCCGTGTCCAGGTATAAC		1163–1143			
GIM-1	GIM-1F	AGAACCTTGACCGAACGCAG	AJ620678	837–856	748	No	26
	GIM-1R	ACTCATGACTCCTCACGAGG		1584–1565			
SIM-1	SIM1-F	TACAAGGGATTCGGCATCG	AY887066	620–638	571	No	104
	SIM1-R	TAATGGCCTGTTCCCATGTG		1190–1171			
Integron PCR	5′ CS	GGCATCCAAGCAGCAAG	M73819	1190–1206	Variable		106
	3′ CS	AAGCAGACTTGACCTGA					

[a] Some primers are outside the coding regions and amplify the entire β-lactamase gene, as indicated; others are internal fragments for diagnostic purposes.

[b] *, NMC primers also amplify NMC-R. Where primers are designated "forward" and "reverse," they were not given names in the referenced study.

[c] "Yes" indicates that entire coding region is amplified; "No" indicates that only a part of the coding region is amplified. *, primers cover the extreme ends of the protein.

of <4 μg/ml (222, 239). It has also been observed that EDTA alone has inhibitory action against some bacteria due to permeabilization of the outer membrane and can lead to false-positive results (27). Etest metallo-β-lactamase detection tests have also yielded false-positive results with OXA-23-producing *A. baumannii* (194).

Carbapenem inactivation assays can be a fast, sensitive method for initial characterization of carbapenem-resistant isolates. The cloverleaf test is a microbiological assay of carbapenemase activity where suspensions of whole cells or and/or an extract of the suspect isolate are tested against imipenem on an agar plate (73). Altered growth of an indicator strain around an imipenem disk is a positive result. One advantage of this test is that enzymes that have very weak car-

bapenemase activity, such as OXA-23 (73) or GES-5 and GES-6 (218), can be detected by this method.

Biochemical and Molecular Tests

Isoelectric focusing (IEF) separates proteins by charge, and detection of β-lactamases is accomplished with the chromogenic cephalosporin nitrocefin (130). Overlay of the gel with EDTA, clavulanic acid, or aztreonam can detect sensitivity of the enzymes to these potential inhibitors, indicating class B, A, or C β-lactamases, respectively. Although IEF results cannot identify a specific β-lactamase, information about isoelectric point and inhibition characteristics can be obtained by this method. IEF is especially valuable for the detection of multiple β-lactamases present in an isolate.

IEF can also be combined with a bioassay to detect the presence of carbapenemases by using an overlay of agar with imipenem and a second overlay with a susceptible indicator organism (103, 192, 241). Growth over an enzyme band indicates a potential carbapenemase. This procedure is especially useful when working with carbapenemases that have poor hydrolysis rates with nitrocefin, such as the metallo-β-lactamases from Aeromonas spp. (241).

Imipenem hydrolysis can most reliably be detected with a spectrophotometric measurement using crude cell extracts or purified β-lactamases (101). If the carbapenemase is a metalloenzyme, a brief incubation with EDTA prior to initiation of the reaction will result in a lower hydrolysis rate. Very weak carbapenemases cannot be detected by this method unless large amounts of extracts are used.

When the presence of a carbapenemase is suspected, PCR is the fastest way to determine which family of β-lactamase is present. Table 7 lists a selection of published primers that have been used with standard PCR technology to detect all of the families and subgroups of carbapenemases known at this time.

Some laboratories have used colony blot hybridizations to efficiently screen large numbers of clinical isolates for carbapenemase genes (121, 240). Hybridization techniques are also used with a Southern blot to determine whether the carbapenemase gene resides on a plasmid or is chromosomal (116).

Ultimately, the identification of the β-lactamase gene requires sequencing of the entire coding region. Cloning of the region around the β-lactamase is usually accomplished with a "shotgun" approach, but a clever degenerate PCR method has also been used successfully to amplify 5′ and 3′ areas surrounding OXA-51 (19). Characterization of a new β-lactamase is not complete until both a molecular sequence is obtained and a functional analysis of the hydrolysis and inhibition profiles is performed with purified protein.

CARBAPENEMASE ORIGINS AND TRANSMISSION

Carbapenem antibiotic design was inspired by the natural product thienamycin, produced by the soil organism Streptomyces cattleya (89). In fact, carbapenems and olivanic acids were some of the most potent naturally occurring β-lactams to be identified from diverse sources in early natural product screening programs (24). Because of the prevalence of these molecules in the soil, it is only logical to expect that enzymes

capable of degrading these β-lactams would be produced by environmental organisms such as Bacillus cereus and Bacillus anthracis, bacteria with well-characterized metallo-β-lactamases that would provide a selective advantage for growth of these environmental species (98, 128). These chromosomal carbapenemases may have evolved initially as a mechanism for bacteria to protect themselves from external threats to their cell wall (20), but in addition these β-lactamases may also play a role in the regulation of cell wall synthesis (151). As described in this review and its many supporting references, the problem of carbapenemase-mediated resistance intensified once genes for these enzymes became associated with acquired genetic determinants. Transmission of carbapenemase genes may occur readily when the gene is located within mobile elements such as plasmids and integrons (82, 133).

Investigators looking for prospective sources of carbapenemase genes have been able to find several in environmental species. The class A carbapenemase SFC-1 was described in an environmental isolate of Serratia fonticola (67). Several of the OXA carbapenemase genes such as OXA-50 and its variants in P. aeruginosa (56, 96), OXA-51-like enzymes in A. baumannii (69, 213), OXA-62 in Pandoraea pnomenusa (192), and OXA-54 and OXA-55 in Shewanella spp. (71, 165) appear to be natural components of their respective bacterial chromosomes. In the case of the OXA genes in A. baumannii, insertion sequences of the ISAba1 type, carrying strong promoters, have been detected upstream of the chromosomal oxacillinase, resulting in increased expression and concomitant carbapenemase resistance (212). Transmission of carbapenemase genes is accelerated when the gene is located within mobile elements such as plasmids and integrons.

In addition to the finding of novel carbapenemases in environmental isolates, enzymes first detected in the clinic are now being found in environmental bacteria. The VIM-2 carbapenemase was found in a Pseudomonas pseudoalcaligenes strain from a hospital wastewater system (180); further examination found two P. aeruginosa strains with this gene. In another interesting study, bacteriophages carrying β-lactamase genes for OXA-type β-lactamases were isolated from sewage, suggesting another vector for transfer of these genes between organisms (140).

While it is not hard to imagine carbapenemase-producing strains finding their way into the sewage system, the discovery of IMI-2 carbapenemases on plasmids in rare E. asburiae isolates from U.S. rivers is harder to explain, although the location of sampling in relation to population centers was not reported (9). It is likely that the circulation of carbapenemase genes proceeds in two directions: environmental sources may provide genetic material as a source of these enzymes, and clinical strains may disperse this information both within the hospital setting and into the surrounding environment.

CONCLUDING REMARKS

Carbapenemase-producing pathogens cause infections that are difficult to treat and have high mortality rates, due to their appearance in multidrug-resistant pathogens such as K. pneumoniae, P. aeruginosa, and Acinetobacter spp. (15, 29, 126). The first descriptions of these enzymes as species-specific chromosomal carbapenemases have more recently been followed by

the appearance of carbapenemase genes that are easily transferred on mobile elements among species. While considered by some to be relatively rare, reports of their occurrence in outbreak settings have steadily increased. Awareness of their entry into a hospital environment is the first step that clinical microbiologists can take to address this problem. Care in detection is needed, because high carbapenem MICs are not always evident. Evaluation of effective antibiotic options and rigorous infection control measures will help in the fight against carbapenemase-producing organisms.

REFERENCES

1. **Aboufaycal, H., H. S. Sader, K. Rolston, L. M. Deshpande, M. Toleman, G. Bodey, I. Raad, and R. N. Jones.** 2007. bla_{VIM-2} and bla_{VIM-7} carbapenemase-producing *Pseudomonas aeruginosa* isolates detected in a tertiary care medical center in the United States: report from the MYSTIC program. J. Clin. Microbiol. **45:**614–615.

2. **Afzal-Shah, M., and D. M. Livermore.** 1998. Worldwide emergence of carbapenem-resistant *Acinetobacter* spp. J. Antimicrob. Chemother. **41:**576–577.

3. **Afzal-Shah, M., N. Woodford, and D. M. Livermore.** 2001. Characterization of OXA-25, OXA-26, and OXA-27, molecular class D β-lactamases associated with carbapenem resistance in clinical isolates of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **45:**583–588.

4. **Alba, J., Y. Ishii, K. Thomson, S. Moland Ellen, and K. Yamaguchi.** 2005. Kinetics study of KPC-3, a plasmid-encoded class A carbapenem-hydrolyzing β-lactamase. Antimicrob. Agents Chemother. **49:**4760–4762.

5. **Ambler, R. P.** 1980. The structure of β-lactamases. Philos. Trans. R. Soc. Lond. B **289:**321–331.

6. **Ambler, R. P., A. F. W. Coulson, J. M. Frere, J. M. Ghuysen, B. Joris, M. Forsman, R. C. Levesque, G. Tiraby, and S. G. Waley.** 1991. A standard numbering scheme for the class A β-lactamases. Biochem. J. **276:**269–270.

7. **Arakawa, Y., M. Murakami, K. Suzuki, H. Ito, R. Wacharotayankun, S. Ohsuka, N. Kato, and M. Ohta.** 1995. A novel integron-like element carrying the metallo-β-lactamase gene bla_{IMP}. Antimicrob. Agents Chemother. **39:**1612–1615.

8. **Arakawa, Y., N. Shibata, K. Shibayama, H. Kurokawa, T. Yagi, H. Fujiwara, and M. Goto.** 2000. Convenient test for screening metallo-β-lactamase-producing gram-negative bacteria by using thiol compounds. J. Clin. Microbiol. **38:**40–43.

9. **Aubron, C., L. Poirel, J. Ash Ronald, and P. Nordmann.** 2005. Carbapenemase-producing *Enterobacteriaceae*, U.S. rivers. Emerg. Infect. Dis. **11:**260–264.

10. **Bahar, G., A. Mazzariol, R. Koncan, A. Mert, R. Fontana, G. M. Rossolini, and G. Cornaglia.** 2004. Detection of VIM-5 metallo-β-lactamase in a *Pseudomonas aeruginosa* clinical isolate from Turkey. J. Antimicrob. Chemother. **54:**282–283.

11. **Bandoh, K., K. Watanabe, Y. Muto, Y. Tanaka, N. Kato, and K. Ueno.** 1992. Conjugal transfer of imipenem resistance in *Bacteroides fragilis*. J. Antibiot. (Tokyo) **45:**542–547.

12. **Bou, G., A. Oliver, and J. Martinez-Beltran.** 2000. OXA-24, a novel class D β-lactamase with carbapenemase activity in an *Acinetobacter baumannii* clinical strain. Antimicrob. Agents Chemother. **44:**1556–1561.

13. **Bradford, P. A., S. Bratu, C. Urban, M. Visalli, N. Mariano, D. Landman, J. J. Rahal, S. Brooks, S. Cebular, and J. Quale.** 2004. Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 β-lactamases in New York City. Clin. Infect. Dis. **39:**55–60.

14. **Bratu, S., D. Landman, M. Alam, E. Tolentino, and J. Quale.** 2005. Detection of KPC carbapenem-hydrolyzing enzymes in *Enterobacter* spp. from Brooklyn, New York. Antimicrob. Agents Chemother. **49:**776–778.

15. **Bratu, S., D. Landman, R. Haag, R. Recco, A. Eramo, M. Alam, and J. Quale.** 2005. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: a new threat to our antibiotic armamentarium. Arch. Intern. Med. **165:**1430–1435.

16. **Bratu, S., M. Mooty, S. Nichani, D. Landman, C. Gullans, B. Pettinato, U. Karumudi, P. Tolaney, and J. Quale.** 2005. Emergence of KPC-possessing *Klebsiella pneumoniae* in Brooklyn, New York: Epidemiology and recommendations for detection. Antimicrob. Agents Chemother. **49:**3018–3020.

17. **Bratu, S., P. Tolaney, U. Karumudi, J. Quale, M. Mooty, S. Nichani, and D. Landman.** 2005. Carbapenemase-producing *Klebsiella pneumoniae* in Brooklyn, N.Y.: molecular epidemiology and in vitro activity of polymyxin B and other agents. J. Antimicrob. Chemother. **56:**128–132.

18. **Brown, S., and S. Amyes.** 2006. OXA β-lactamases in *Acinetobacter*: the story so far. J. Antimicrob. Chemother. **57:**1–3.

19. **Brown, S., H. K. Young, and S. G. B. Amyes.** 2005. Characterisation of OXA-51, a novel class D carbapenemase found in genetically unrelated

20. **Bush, K.** 1997. The evoution of β-lactamases, p. 152–166. *In* D. J. Chadwick and J. Goode (ed.), Antibiotic resistance: origins, evolution, selection and spread, vol. 207. John Wiley & Sons, Chichester, United Kingdom.

21. **Bush, K.** 1988. Recent developments in β-lactamase research and their implications for the future. Rev. Infect. Dis. **10:**681–690; 739–743.

22. **Bush, K., G. A. Jacoby, and A. A. Medeiros.** 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. **39:**1211–1233.

23. **Bush, K., and R. B. Sykes.** 1987. Characterization and epidemiology of β-lactamases. Elsevier Science Publishers BV, Philadelphia, PA.

24. **Butterworth, D., M. Cole, G. Hanscomb, and G. N. Rolinson.** 1979. Olivanic acids, a family of β-lactam antibiotics with β-lactamase inhibitory properties produced by *Streptomyces* species. I. Detection, properties and fermentation studies. J. Antibiot. **32:**287–294.

25. **Castanheira, M., R. E. Mendes, T. R. Walsh, A. C. Gales, and R. N. Jones.** 2004. Emergence of the extended-spectrum β-lactamase GES-1 in a *Pseudomonas aeruginosa* strain from Brazil: report from the SENTRY antimicrobial surveillance program. Antimicrob. Agents Chemother. **48:**2344–2345.

26. **Castanheira, M., M. A. Toleman, R. N. Jones, F. J. Schmidt, and T. R. Walsh.** 2004. Molecular characterization of a β-lactamase gene, bla_{GIM-1}, encoding a new subclass of metallo-β-lactamase. Antimicrob. Agents Chemother. **48:**4654–4661.

27. **Chu, Y. W., T. K. M. Cheung, J. Y. W. Ngan, and K. M. Kam.** 2005. EDTA susceptibility leading to false detection of metallo-β-lactamase in *Pseudomonas aeruginosa* by Etest and an imipenem-EDTA disk method. Int. J. Antimicrob. Agents **26:**340–341.

28. **Coelho, J., N. Woodford, M. Afzal-Shah, and D. Livermore.** 2006. Occurrence of OXA-58-like carbapenemases in *Acinetobacter* spp. collected over 10 years in three continents. Antimicrob. Agents Chemother. **50:**756–758.

29. **Coelho, J. M., J. F. Turton, M. E. Kaufmann, J. Glover, N. Woodford, M. Warner, M.-F. Palepou, R. Pike, T. L. Pitt, B. C. Patel, and D. M. Livermore.** 2006. Occurrence of carbapenem-resistant *Acinetobacter baumannii* clones at multiple hospitals in London and southeast England. J. Clin. Microbiol. **44:**3623–3627.

30. **Conceicao, T., A. Brizio, A. Duarte, and R. Barros.** 2005. First isolation of bla_{VIM-2} in *Klebsiella oxytoca* clinical isolates from Portugal. Antimicrob. Agents Chemother. **49:**476.

31. **Concha, N. O., C. A. Janson, P. Rowling, S. Pearson, C. A. Cheever, B. P. Clarke, C. Lewis, M. Galleni, J. M. Frere, D. J. Payne, J. H. Bateson, and S. S. Abdel-Meguid.** 2000. Crystal structure of the IMP-1 metallo β-lactamase from *Pseudomonas aeruginosa* and its complex with a mercaptocarboxylate inhibitor: binding determinants of a potent, broad-spectrum inhibitor. Biochemistry **39:**4288–4298.

32. **Concha, N. O., B. A. Rasmussen, K. Bush, and O. Herzberg.** 1996. Crystal structure of the wide-spectrum binuclear zinc β-lactamase from *Bacteroides fragilis*. Structure **4:**823–836.

33. **Correia, M., F. Boavida, F. Grosso, M. J. Salgado, L. M. Lito, J. M. Cristino, S. Mendo, and A. Duarte.** 2003. Molecular characterization of a new class 3 integron in *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **47:**2838–2843.

34. **Costello, A. L., N. P. Sharma, K.-W. Yang, M. W. Crowder, and D. L. Tierney.** 2006. X-ray absorption spectroscopy of the zinc-binding sites in the class B2 metallo-β-lactamase ImiS from *Aeromonas veronii* bv. *sobria*. Biochemistry **45:**13650–13658.

35. **Couture, F., J. Lachapelle, and R. C. Levesque.** 1992. Phylogeny of LCR-1 and OXA-5 with class A and class D β-lactamases. Mol. Microbiol. **6:**1693–1705.

36. **Crespo, M. P., N. Woodford, A. Sinclair, M. E. Kaufmann, J. Turton, J. Glover, J. D. Velez, C. R. Castaneda, M. Recalde, and D. M. Livermore.** 2004. Outbreak of carbapenem-resistant *Pseudomonas aeruginosa* producing VIM-8, a novel metallo-β-lactamase, in a tertiary care center in Cali, Colombia. J. Clin. Microbiol. **42:**5094–5101.

37. **Dalla-Costa, L. M., J. M. Coelho, H. A. P. H. M. Souza, M. E. S. Castro, C. J. N. Stier, K. L. Bragagnolo, A. Rea-Neto, S. R. Penteado-Filho, D. M. Livermore, and N. Woodford.** 2003. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the OXA-23 enzyme in Curitiba, Brazil. J. Clin. Microbiol. **41:**3403–3406.

38. **Danel, F., L. M. C. Hall, D. Gur, and D. M. Livermore.** 1997. OXA-15, an extended-spectrum variant of OXA-2 β-lactamase, isolated from a *Pseudomonas aeruginosa* strain. Antimicrob. Agents Chemother. **41:**785–790.

39. **Da Silva, G. J., M. Correia, C. Vital, G. Ribeiro, J. C. Sousa, R. Leitao, L. Peixe, and A. Duarte.** 2002. Molecular characterization of bla$_{IMP-5}$, a new integron-borne metallo-β-lactamase gene from an *Acinetobacter baumannii* nosocomial isolate in Portugal. FEMS Microbiol. Lett. **215:**33–39.

40. **Da Silva, G. J., S. Quinteira, E. Bertolo, J. C. Sousa, L. Gallego, A. Duarte, and L. Peixe.** 2004. Long-term dissemination of an OXA-40 carbapenemase-producing *Acinetobacter baumannii* clone in the Iberian Peninsula. J. Antimicrob. Chemother. **54:**255–258.

41. **Donald, H. M., W. Scaife, S. G. Amyes, and H. K. Young.** 2000. Sequence

clinical strains of *Acinetobacter baumannii* from Argentina. Clin. Microbiol. Infect. **11:**15–23.

analysis of ARI-1, a novel OXA β-lactamase, responsible for imipenem resistance in *Acinetobacter baumannii* 6B92. Antimicrob. Agents Chemother. **44:**196–199.

42. **Duarte, A., F. Boavida, F. Grosso, M. Correia, L. M. Lito, J. M. Cristino, and M. J. Salgado.** 2003. Outbreak of GES-1 β-lactamase-producing multidrug-resistant *Klebsiella pneumoniae* in a university hospital in Lisbon, Portugal. Antimicrob. Agents Chemother. **47:**1481–1482.

43. **Espedido, B., J. Iredell, L. Thomas, and A. Zelynski.** 2005. Wide dissemination of a carbapenemase plasmid among gram-negative bacteria: implications of the variable phenotype. J. Clin. Microbiol. **43:**4918–4919.

44. **Fabiane, S. M., M. K. Sohi, T. Wan, D. J. Payne, J. H. Bateson, T. Mitchell, and B. J. Sutton.** 1998. Crystal structure of the zinc-dependent β-lactamase from *Bacillus cereus* at 1.9 Å resolution: binuclear active site with features of a mononuclear enzyme. Biochemistry **37:**12404–12411.

45. **Fiett, J., A. Baraniak, A. Mrowka, M. Fleischer, Z. Drulis-Kawa, L. Naumiuk, A. Samet, W. Hryniewicz, and M. Gniadkowski.** 2006. Molecular epidemiology of acquired-metallo-β-lactamase-producing bacteria in Poland. Antimicrob. Agents Chemother. **50:**880–886.

46. **Frere, J. M., M. Galleni, K. Bush, and O. Dideberg.** 2005. Is it necessary to change the classification of β-lactamases? J. Antimicrob. Chemother. **55:**1051–1053.

47. **Gacar, G. G., K. Midilli, F. Kolayli, K. Ergen, S. Gundes, S. Hosoglu, A. Karadenizli, and H. Vahaboglu.** 2005. Genetic and enzymatic properties of metallo-β-lactamase VIM-5 from a clinical isolate of *Enterobacter cloacae*. Antimicrob. Agents Chemother. **49:**4400–4403.

48. **Galani, I., M. Souli, Z. Chryssouli, K. Orlandou, and H. Giamarellou.** 2005. Characterization of a new integron containing bla$_{VIM-1}$ and aac(6′)-IIc in an *Enterobacter cloacae* clinical isolate from Greece. J. Antimicrob. Chemother. **55:**634–638.

49. **Gales, A. C., D. J. Biedenbach, P. Winokur, D. M. Hacek, M. A. Pfaller, and R. N. Jones.** 2001. Carbapenem-resistant *Serratia marcescens* isolates producing Bush group 2f β-lactamase (SME-1) in the United States: results from the MYSTIC Programme. Diagn. Microbiol. Infect. Dis. **39:**125–127.

50. **Galleni, M., J. Lamotte-Brasseur, G. M. Rossolini, J. Spencer, O. Dideberg, and J. M. Frere.** 2001. Standard numbering scheme for class B β-lactamases. Antimicrob. Agents Chemother. **45:**660–663.

51. **Garau, G., I. Garcia-Saez, C. Bebrone, C. Anne, P. Mercuri, M. Galleni, J.-M. Frere, and O. Dideberg.** 2004. Update of the standard numbering scheme for class B β-lactamases. Antimicrob. Agents Chemother. **48:**2347–2349.

52. **Garcia-Saez, I., J. Hopkins, C. Papamicael, N. Franceschini, G. Amicosante, G. M. Rossolini, M. Galleni, J.-M. Frere, and O. Dideberg.** 2003. The 1.5-Å structure of *Chryseobacterium meningosepticum* zinc β-lactamase in complex with the inhibitor, D-captopril. J. Biol. Chem. **278:**23868–23873.

53. **Giakkoupi, P., L. S. Tzouvelekis, A. Tsakris, V. Loukova, D. Sofianou, and E. Tzelepi.** 2000. IBC-1, a novel integron-associated class A β-lactamase with extended-spectrum properties produced by an *Enterobacter cloacae* clinical strain. Antimicrob. Agents Chemother. **44:**2247–2253.

54. **Giakkoupi, P., A. Xanthaki, M. Kanelopoulou, A. Vlahaki, V. Miriagou, S. Kontou, E. Papafraggas, H. Malamou-Lada, L. S. Tzouvelekis, N. J. Legakis, and A. C. Vatopoulos.** 2003. VIM-1 metallo-β-lactamase-producing *Klebsiella pneumoniae* strains in Greek hospitals. J. Clin. Microbiol. **41:**3893–3896.

55. **Gibb, A. P., C. Tribuddharat, R. A. Moore, T. J. Louie, W. Krulicki, D. M. Livermore, M.-F. I. Palepou, and N. Woodford.** 2002. Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new bla$_{IMP}$ allele, bla$_{IMP-7}$. Antimicrob. Agents Chemother. **46:**255–258.

56. **Girlich, D., T. Naas, and P. Nordmann.** 2004. Biochemical characterization of the naturally occurring oxacillinase OXA-50 of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **48:**2043–2048.

57. **Girlich, D., T. Naas, and P. Nordmann.** 2004. OXA-60, a chromosomal, inducible, and imipenem-hydrolyzing class D β-lactamase from *Ralstonia pickettii*. Antimicrob. Agents Chemother. **48:**4217–4225.

58. **Guerin, F., C. Henegar, G. Spiridon, O. Launay, D. Salmon-Ceron, and C. Poyart.** 2005. Bacterial prostatitis due to *Pseudomonas aeruginosa* harboring the bla$_{VIM-2}$ metallo-β-lactamase gene from Saudi Arabia. J. Antimicrob. Chemother. **56:**601–602.

59. **Gupta, V., P. Datta, and J. Chander.** 2006. Prevalence of metallo-β-lactamase (MBL) producing *Pseudomonas* spp. and *Acinetobacter* spp. in a tertiary care hospital in India. J. Infect. **52:**311–314.

60. **Hall, B. G., and M. Barlow.** 2005. Revised Ambler classification of β-lactamases. J. Antimicrob. Chemother. **55:**1050–1051.

61. **Hall, L. M. C., D. M. Livermore, D. Gur, M. Akova, and H. E. Akalin.** 1993. OXA-11, an extended-spectrum variant of OXA-10 (PSE-2) β-lactamase from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **37:**1637–1644.

62. **Hanson, N. D., A. Hossain, L. Buck, S. Moland Ellen, and S. Thomson Kenneth.** 2006. First occurrence of a *Pseudomonas aeruginosa* isolate in the United States producing an IMP metallo-β-lactamase, IMP-18. Antimicrob. Agents Chemother. **50:**2272–2273.

63. **Hawkey, P. M., J. Xiong, H. Ye, H. Li, and F. H. M'Zali.** 2001. Occurrence of a new metallo-β-lactamase IMP-4 carried on a conjugative plasmid in

64. **Hayes, M. V., C. J. Thomson, and S. G. B. Amyes.** 1996. The "hidden" carbapenemase of *Aeromonas hydrophila*. J. Antimicrob. Chemother. **37:**33–44.

65. **Hayes, M. V., C. J. Thomson, and S. G. B. Amyes.** 1994. Three β-lactamases isolated from *Aeromonas salmonicida*, including a carbapenemase not detectable by conventional methods. Eur. J. Clin. Microbiol. Infect. Dis. **13:**805–811.

66. **Henrichfreise, B., I. Wiegand, K. J. Sherwood, and B. Wiedemann.** 2005. Detection of VIM-2 metallo-β-lactamase in *Pseudomonas aeruginosa* from Germany. Antimicrob. Agents Chemother. **49:**1668–1669.

67. **Henriques, I., A. Moura, A. Alves, M. J. Saavedra, and A. Correia.** 2004. Molecular characterization of a carbapenem-hydrolyzing class A β-lactamase, SFC-1, from *Serratia fonticola* UTAD54. Antimicrob. Agents Chemother. **48:**2321–2324.

68. **Heritier, C., A. Dubouix, L. Poirel, N. Marty, and P. Nordmann.** 2005. A nosocomial outbreak of *Acinetobacter baumannii* isolates expressing the carbapenem-hydrolysing oxacillinase OXA-58. J. Antimicrob. Chemother. **55:**115–118.

69. **Heritier, C., L. Poirel, P.-E. Fournier, J.-M. Claverie, D. Raoult, and P. Nordmann.** 2005. Characterization of the naturally occurring oxacillinase of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **49:**4174–4179.

70. **Heritier, C., L. Poirel, T. Lambert, and P. Nordmann.** 2005. Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **49:**3198–3202.

71. **Heritier, C., L. Poirel, and P. Nordmann.** 2004. Genetic and biochemical characterization of a chromosome-encoded carbapenem-hydrolyzing Ambler class D β-lactamase from *Shewanella algae*. Antimicrob. Agents Chemother. **48:**1670–1675.

72. **Hirakata, Y., K. Izumikawa, T. Yamaguchi, H. Takemura, H. Tanaka, R. Yoshida, J. Matsuda, M. Nakano, K. Tomono, S. Maesaki, M. Kaku, Y. Yamada, S. Kamihira, and S. Kohno.** 1998. Rapid detection and evaluation of clinical characteristics of emerging multiple-drug-resistant gram-negative rods carrying the metallo-β-lactamase gene bla$_{IMP}$. Antimicrob. Agents Chemother. **42:**2006–2011.

73. **Hornstein, M., C. Sautjeau-Rostoker, J. Peduzzi, A. Vessieres, L. T. Hong, M. Barthelemy, M. Scavizzi, and R. Labia.** 1997. Oxacillin-hydrolyzing β-lactamase involved in resistance to imipenem in *Acinetobacter baumannii*. FEMS Microbiol. Lett. **153:**333–339.

74. **Hossain, A., M. J. Ferraro, R. M. Pino, R. B. Dew III, E. S. Moland, T. J. Lockhart, K. S. Thomson, R. V. Goering, and N. D. Hanson.** 2004. Plasmid-mediated carbapenem-hydrolyzing enzyme KPC-2 in an *Enterobacter* sp. Antimicrob. Agents Chemother. **48:**4438–4440.

75. **Hujer, K. M., A. M. Hujer, E. A. Hulten, S. Bajaksouzian, J. M. Adams, C. J. Donskey, D. J. Ecker, C. Massire, M. W. Eshoo, R. Sampath, J. M. Thomson, P. N. Rather, D. W. Craft, J. T. Fishbain, A. J. Ewell, M. R. Jacobs, D. L. Paterson, and R. A. Bonomo.** 2006. Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter* sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrob. Agents Chemother. **50:**4114–4123.

76. **Huovinen, P., S. Huovinen, and G. A. Jacoby.** 1988. Sequence of PSE-2 β-lactamase. Antimicrob. Agents Chemother. **32:**134–136.

77. **Hussain, M., A. Carlino, M. J. Madonna, and J. O. Lampen.** 1985. Cloning and sequencing of the metallothioprotein β-lactamase II gene of *Bacillus cereus* 569/H in *Escherichia coli*. J. Bacteriol. **164:**223–229.

78. **Iaconis, J. P., and C. C. Sanders.** 1990. Purification and characterization of inducible β-lactamases in *Aeromonas* spp. Antimicrob. Agents Chemother. **34:**44–51.

79. **Ikonomidis, A., D. Tokatlidou, I. Kristo, D. Sofianou, A. Tsakris, P. Mantzana, S. Pournaras, and A. N. Maniatis.** 2005. Outbreaks in distinct regions due to a single *Klebsiella pneumoniae* clone carrying a bla$_{VIM-1}$ metallo-β-lactamase gene. J. Clin. Microbiol. **43:**5344–5347.

80. **Ito, H., Y. Arakawa, S. Ohsuka, R. Wacharotayankun, N. Kato, and M. Ohta.** 1995. Plasmid-mediated dissemination of the metallo-β-lactamase gene bla$_{IMP}$ among clinically isolated strains of *Serratia marcescens*. Antimicrob. Agents Chemother. **39:**824–829.

81. **Iyobe, S., H. Kusadokoro, J. Ozaki, N. Matsumura, S. Minami, S. Haruta, T. Sawai, and K. O'Hara.** 2000. Amino acid substitutions in a variant of IMP-1 metallo-β-lactamase. Antimicrob. Agents Chemother. **44:**2023–2027.

82. **Jacoby, G., and K. Bush.** 2005. β-Lactam resistance in the 21st century, p. 53–65. *In* D. G. White, M. N. Alekshun, and P. F. McDermott (ed.), Frontiers in antimicrobial resistance: a tribute to Stuart B. Levy. ASM Press, Washington, DC.

83. **Jacoby, G. A.** 2006. β-Lactamase nomenclature. Antimicrob. Agents Chemother. **50:**1123–1129.

84. **Jaurin, B., and T. Grundstrom.** 1981. *ampC* cephalosporinase of *Escherichia coli* K-12 has a different evolutionary origin from that of β-lactamases of the penicillinase type. Proc. Natl. Acad. Sci. USA **78:**4897–4901.

85. **Jeon, B.-C., S. H. Jeong, I. K. Bae, S. B. Kwon, K. Lee, D. Young, J. H. Lee,**

J. S. Song, and S. H. Lee. 2005. Investigation of a nosocomial outbreak of imipenem-resistant *Acinetobacter baumannii* producing the OXA-23 β-lactamase in Korea. J. Clin. Microbiol. **43:**2241–2245.

86. **Jeong, S. H., I. K. Bae, D. Kim, S. G. Hong, J. S. Song, J. H. Lee, and S. H. Lee.** 2005. First outbreak of *Klebsiella pneumoniae* clinical isolates producing GES-5 and SHV-12 extended-spectrum β-lactamases in Korea. Antimicrob. Agents Chemother. **49:**4809–4810.

87. **Jeong, S. H., K. Lee, Y. Chong, J. H. Yum, S. H. Lee, H. J. Choi, J. M. Kim, K. H. Park, B. H. Han, S. W. Lee, and T. S. Jeong.** 2003. Characterization of a new integron containing VIM-2, a metallo-β-lactamase gene cassette, in a clinical isolate of *Enterobacter cloacae*. J. Antimicrob. Chemother. **51:**397–400.

88. **Jones, R. N., D. J. Biedenbach, H. S. Sader, T. R. Fritsche, M. A. Toleman, and T. R. Walsh.** 2005. Emerging epidemic of metallo-β-lactamase-mediated resistances. Diagn. Microbiol. Infect. Dis. **51:**77–84.

89. **Kahan, J. S., F. M. Kahan, R. Goegelman, S. A. Currie, M. Jackson, E. O. Stapley, T. W. Miller, A. K. Miller, D. Hendlin, S. Mochales, S. Hernandez, H. B. Woodruff, and J. Birnbaum.** 1979. Thienamycin, a new β-lactam antibiotic. I. Discovery, taxonomy, isolation and physical properties. J. Antibiot. **32:**1–12.

90. **Kartali, G., E. Tzelepi, S. Pournaras, C. Kontopoulou, F. Kontos, D. Sofianou, A. N. Maniatis, and A. Tsakris.** 2002. Outbreak of infections caused by *Enterobacter cloacae* producing the integron-associated β-lactamase IBC-1 in a neonatal intensive care unit of a Greek hospital. Antimicrob. Agents Chemother. **46:**1577–1580.

91. **Kassis-Chikhani, N., D. Decre, V. Gautier, B. Burghoffer, F. Saliba, D. Mathieu, D. Samuel, D. Castaing, J.-C. Petit, E. Dussaix, and G. Arlet.** 2006. First outbreak of multidrug-resistant *Klebsiella pneumoniae* carrying bla$_{VIM-1}$ and bla$_{SHV-5}$ in a French university hospital. J. Antimicrob. Chemother. **57:**142–145.

92. **Ke, W., C. R. Bethel, J. M. Thomson, R. A. Bonomo, and F. van den Akker.** 2007. Crystal structure of KPC-2: insights into carbapenemase activity in class A β-lactamases. Biochemistry **46:**5732–5740.

93. **Kiffer, C. R. V., C. Mendes, J. L. Kuti, and D. P. Nicolau.** 2004. Pharmacodynamic comparisons of antimicrobials against nosocomial isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* from the MYSTIC surveillance program: the OPTAMA Program, South America 2002. Diagn. Microbiol. Infect. Dis. **49:**109–116.

94. **Kimura, S., J. Alba, K. Shiroto, R. Sano, Y. Niki, S. Maesaki, K. Akizawa, M. Kaku, Y. Watanuki, Y. Ishii, and K. Yamaguchi.** 2005. Clonal diversity of metallo-β-lactamase-possessing *Pseudomonas aeruginosa* in geographically diverse regions of Japan. J. Clin. Microbiol. **43:**458–461.

95. **Koh, T. H., G. S. Babini, N. Woodford, L. H. Sng, L. M. Hall, and D. M. Livermore.** 1999. Carbapenem-hydrolysing IMP-1 β-lactamase in *Klebsiella pneumoniae* from Singapore. Lancet **353:**2162.

96. **Kong, K.-F., S. R. Jayawardena, A. Del Puerto, L. Wiehlmann, U. Laabs, B. Tummler, and K. Mathee.** 2005. Characterization of poxB, a chromosomal-encoded *Pseudomonas aeruginosa* oxacillinase. Gene **358:**82–92.

97. **Ktari, S., G. Arlet, B. Mnif, V. Gautier, F. Mahjoubi, M. Ben Jmeaa, M. Bouaziz, and A. Hammami.** 2006. Emergence of multidrug-resistant *Klebsiella pneumoniae* isolates producing VIM-4 metallo-β-lactamase, CTX-M-15 extended-spectrum β-lactamase, and CMY-4 AmpC β-lactamase in a Tunisian university hospital. Antimicrob. Agents Chemother. **50:**4198–4201.

98. **Kuwabara, S., and E. P. Abraham.** 1967. Some properties of two extracellular β-lactamases from *Bacillus cereus* 569/H. Biochem. J. **103:**27C–30C.

99. **Reference deleted.**

100. **Lagatolla, C., E. A. Tonin, C. Monti-Bragadin, L. Dolzani, F. Gombac, C. Bearzi, E. Edalucci, F. Gionechetti, and G. M. Rossolini.** 2004. Endemic carbapenem-resistant *Pseudomonas aeruginosa* with acquired metallo-β-lactamase determinants in European hospital. Emerg. Infect. Dis. **10:**535–538.

101. **Lauretti, L., M. L. Riccio, A. Mazzariol, G. Cornaglia, G. Amicosante, R. Fontana, and G. M. Rossolini.** 1999. Cloning and characterization of bla$_{VIM}$, a new integron-borne metallo-β-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. Antimicrob. Agents Chemother. **43:**1584–1590.

102. **Ledent, P., X. Raquet, B. Joris, J. Van Beeumen, and J. M. Frere.** 1993. A comparative study of class-D β-lactamases. Biochem. J. **292:**555–562.

103. **Lee, K., J. B. Lim, J. H. Yum, D. Yong, Y. Chong, J. M. Kim, and D. M. Livermore.** 2002. bla$_{VIM-2}$ cassette-containing novel integrons in metallo-β-lactamase-producing *Pseudomonas aeruginosa* and *Pseudomonas putida* isolates disseminated in a Korean hospital. Antimicrob. Agents Chemother. **46:**1053–1058.

104. **Lee, K., J. H. Yum, D. Yong, H. M. Lee, H. D. Kim, J.-D. Docquier, G. M. Rossolini, and Y. Chong.** 2005. Novel acquired metallo-β-lactamase gene, bla$_{SIM-1}$, in a class 1 integron from *Acinetobacter baumannii* clinical isolates from Korea. Antimicrob. Agents Chemother. **49:**4485–4491.

105. **Lee, S. H., and S. H. Jeong.** 2005. Nomenclature of GES-type extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **49:**2148.

106. **Levesque, C., L. Piche, C. Larose, and P. H. Roy.** 1995. PCR mapping of

107. integrons reveals several novel combinations of resistance genes. Antimicrob. Agents Chemother. **39:**185–191.

107. **Libisch, B., M. Gacs, K. Csiszar, M. Muzslay, L. Rokusz, and M. Fuezi.** 2004. Isolation of an integron-borne bla$_{VIM-4}$ type metallo-β-lactamase gene from a carbapenem-resistant *Pseudomonas aeruginosa* clinical isolate in Hungary. Antimicrob. Agents Chemother. **48:**3576–3578.

108. **Libisch, B., M. Muzslay, M. Gacs, J. Minarovits, M. Knausz, J. Watine, G. Ternak, E. Kenez, I. Kustos, L. Rokusz, K. Szeles, B. Balogh, and M. Fuzi.** 2006. Molecular epidemiology of VIM-4 metallo-β-lactamase-producing *Pseudomonas* sp. isolates in Hungary. Antimicrob. Agents Chemother. **50:**4220–4223.

109. **Lim, H. M., J. J. Pene, and R. W. Shaw.** 1988. Cloning, nucleotide sequence, and expression of the *Bacillus cereus* 5/B/6 β-lactamase II structural gene. J. Bacteriol. **170:**2873–2878.

110. **Lincopan, N., J. A. McCulloch, C. Reinert, V. C. Cassettari, A. C. Gales, and E. M. Mamizuka.** 2005. First isolation of metallo-β-lactamase-producing multiresistant *Klebsiella pneumoniae* from a patient in Brazil. J. Clin. Microbiol. **43:**516–519.

111. **Liu, S. Y., J. Y. Lin, C. Chu, L. H. Su, T. Y. Lin, and C. H. Chiu.** 2006. Integron-associated imipenem resistance in *Acinetobacter baumannii* isolated from a regional hospital in Taiwan. Int. J. Antimicrob. Agents. **27:**81–84.

112. **Livermore, D. M.** 2002. The impact of carbapenemases on antimicrobial development and therapy. Curr. Opin. Investig. Drugs **3:**218–224.

113. **Livermore, D. M.** 2001. Of *Pseudomonas*, porins, pumps and carbapenems. J. Antimicrob. Chemother. **47:**247–250.

114. **Livermore, D. M., and N. Woodford.** 2006. The β-lactamase threat in *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter*. Trends Microbiol. **14:**413–420.

115. **Lolans, K., A. M. Queenan, K. Bush, A. Sahud, and J. P. Quinn.** 2005. First nosocomial outbreak of *Pseudomonas aeruginosa* producing an integron-borne metallo-β-lactamase (VIM-2) in the United States. Antimicrob. Agents Chemother. **49:**3538–3540.

116. **Lolans, K., T. W. Rice, L. S. Munoz-Price, and J. P. Quinn.** 2006. Multicity outbreak of carbapenem-resistant *Acinetobacter baumannii* isolates producing the carbapenemase OXA-40. Antimicrob. Agents Chemother. **50:**2941–2945.

117. **Loli, A., L. S. Tzouvelekis, E. Tzelepi, A. Carattoli, A. C. Vatopoulos, P. T. Tassios, and V. Miriagou.** 2006. Sources of diversity of carbapenem resistance levels in *Klebsiella pneumoniae* carrying bla$_{VIM-1}$. J. Antimicrob. Chemother. **58:**669–672.

118. **Lomaestro, B. M., E. H. Tobin, W. Shang, and T. Gootz.** 2006. The spread of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* to upstate New York. Clin. Infect. Dis. **43:**e26–28.

119. **Lopez-Otsoa, F., L. Gallego, K. J. Towner, L. Tysall, N. Woodford, and D. M. Livermore.** 2002. Endemic carbapenem resistance associated with OXA-40 carbapenemase among *Acinetobacter baumannii* isolates from a hospital in northern Spain. J. Clin. Microbiol. **40:**4741–4743.

120. **Luzzaro, F., J.-D. Docquier, C. Colinon, A. Endimiani, G. Lombardi, G. Amicosante, G. M. Rossolini, and A. Toniolo.** 2004. Emergence in *Klebsiella pneumoniae* and *Enterobacter cloacae* clinical isolates of the VIM-4 metallo-β-lactamase encoded by a conjugative plasmid. Antimicrob. Agents Chemother. **48:**648–650.

121. **Luzzaro, F., A. Endimiani, J.-D. Docquier, C. Mugnaioli, M. Bonsignori, G. Amicosante, G. M. Rossolini, and A. Toniolo.** 2004. Prevalence and characterization of metallo-β-lactamases in clinical isolates of *Pseudomonas aeruginosa*. Diagn. Microbiol. Infect. Dis. **48:**131–135.

122. **Majiduddin, F. K., and T. Palzkill.** 2005. Amino acid residues that contribute to substrate specificity of class A β-lactamase SME-1. Antimicrob. Agents Chemother. **49:**3421–3427.

123. **Majiduddin, F. K., and T. Palzkill.** 2003. Amino acid sequence requirements at residues 69 and 238 for the SME-1 β-lactamase to confer resistance to β-lactam antibiotics. Antimicrob. Agents Chemother. **47:**1062–1067.

124. **Mariotte-Boyer, S., M. H. Nicolas-Chanoine, and R. Labia.** 1996. A kinetic study of NMC-A β-lactamase, an Ambler class A carbapenemase also hydrolyzing cephamycins. FEMS Microbiol. Lett. **143:**29–33.

125. **Marque, S., L. Poirel, C. Heritier, S. Brisse, M. D. Blasco, R. Filip, G. Coman, T. Naas, and P. Nordmann.** 2005. Regional occurrence of plasmid-mediated carbapenem-hydrolyzing oxacillinase OXA-58 in *Acinetobacter* spp. in Europe. J. Clin. Microbiol. **43:**4885–4888.

126. **Marra, A. R., C. A. P. Pereira, A. C. Gales, L. C. Menezes, R. G. R. Cal, J. M. A. de Souza, M. B. Edmond, C. Faro, and S. B. Wey.** 2006. Bloodstream infections with metallo-β-lactamase-producing *Pseudomonas aeruginosa*: epidemiology, microbiology, and clinical outcomes. Antimicrob. Agents Chemother. **50:**388–390.

127. **Massova, I., and S. Mobashery.** 1998. Kinship and diversification of bacterial penicillin-binding proteins and β-lactamases. Antimicrob. Agents Chemother. **42:**1–17.

128. **Materon, I. C., A. M. Queenan, T. M. Koehler, K. Bush, and T. Palzkill.** 2003. Biochemical characterization of β-lactamases Bla1 and Bla2 from *Bacillus anthracis*. Antimicrob. Agents Chemother. **47:**2040–2042.

129. **Matthew, M.** 1979. Plasmid mediated β-lactamases of gram-negative bacteria: properties and distribution. J. Antimicrob. Chemother. **5:**349–358.

130. **Matthew, M., A. M. Harris, M. J. Marshall, and G. W. Ross.** 1975. The use of analytical isoelectric focusing for detection and identification of β-lactamases. J. Gen. Microbiol. **88:**169–178.

131. **Maveyraud, L., D. Golemi-Kotra, A. Ishiwata, O. Meroueh, S. Mobashery, and J.-P. Samama.** 2002. High-resolution X-ray structure of an acyl-enzyme species for the class D OXA-10 β-lactamase. J. Am. Chem. Soc. **124:**2461–2465.

132. **Medeiros, A. A.** 1984. β-Lactamases. Br. Med. Bull. **40:**18–27.

133. **Medeiros, A. A.** 1997. Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics. Clinic. Infect. Dis. **24:**S19–S45.

134. **Medeiros, A. A., and R. S. Hare.** 1986. β-Lactamase-mediated resistance to penems and carbapenems amongst *Enterobacteriaceae*, abstr. 116. 26th Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.

135. **Meyer, K. S., C. Urban, J. A. Eagan, B. J. Berger, and J. J. Rahal.** 1993. Nosocomial outbreak of *Klebsiella* infection resistant to late-generation cephalosporins. Ann. Intern. Med. **119:**353–358.

136. **Miriagou, V., E. Tzelepi, D. Gianneli, and L. S. Tzouvelekis.** 2003. *Escherichia coli* with a self-transferable, multiresistant plasmid coding for metallo-β-lactamase VIM-1. Antimicrob. Agents Chemother. **47:**395–397.

137. **Miriagou, V., L. S. Tzouvelekis, S. Rossiter, E. Tzelepi, F. J. Angulo, and J. M. Whichard.** 2003. Imipenem resistance in a *Salmonella* clinical strain due to plasmid-mediated class A carbapenemase KPC-2. Antimicrob. Agents Chemother. **47:**1297–1300.

138. **Moland, E. S., N. D. Hanson, V. L. Herrera, J. A. Black, T. J. Lockhart, A. Hossain, J. A. Johnson, R. V. Goering, and K. S. Thomson.** 2003. Plasmid-mediated, carbapenem-hydrolysing β-lactamase, KPC-2, in *Klebsiella pneumoniae* isolates. J. Antimicrob. Chemother. **51:**711–714.

139. **Mourey, L., K. Miyashita, P. Swaren, A. Bulychev, J.-P. Samama, and S. Mobashery.** 1998. Inhibition of the NMC-A β-lactamase by a penicillanic acid derivative and the structural bases for the increase in substrate profile of this antibiotic resistance enzyme. J. Am. Chem. Soc. **120:**9382–9383.

140. **Muniesa, M., A. Garcia, E. Miro, B. Mirelis, G. Prats, J. Jofre, and F. Navarro.** 2004. Bacteriophages and diffusion of β-lactamase genes. Emerg. Infect. Dis. **10:**1134–1137.

141. **Murphy, T. A., L. E. Catto, S. E. Halford, A. T. Hadfield, W. Minor, T. R. Walsh, and J. Spencer.** 2006. Crystal structure of *Pseudomonas aeruginosa* SPM-1 provides insights into variable zinc affinity of metallo-β-lactamases. J. Mol. Biol. **357:**890–903.

142. **Naas, T., M. Levy, C. Hirschauer, H. Marchandin, and P. Nordmann.** 2005. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the carbapenemase OXA-23 in a tertiary care hospital of Papeete, French Polynesia. J. Clin. Microbiol. **43:**4826–4829.

143. **Naas, T., and P. Nordmann.** 1994. Analysis of a carbapenem-hydrolyzing class A β-lactamase from *Enterobacter cloacae* and of its LysR-type regulatory protein. Proc. Natl. Acad. Sci. USA **91:**7693–7697.

144. **Naas, T., and P. Nordmann.** 1999. OXA-type β-lactamases. Curr. Pharm. Des. **5:**865–879.

145. **Naas, T., P. Nordmann, G. Vedel, and C. Poyart.** 2005. Plasmid-mediated carbapenem-hydrolyzing β-lactamase KPC in a *Klebsiella pneumoniae* isolate from France. Antimicrob. Agents Chemother. **49:**4423–4424.

146. **Naas, T., L. Vandel, W. Sougakoff, D. M. Livermore, and P. Nordmann.** 1994. Cloning and sequence analysis of the gene for a carbapenem-hydrolyzing class A β-lactamase, Sme-1, from *Serratia marcescens* S6. Antimicrob. Agents Chemother. **38:**1262–1270.

147. **Navon-Venezia, S., I. Chmelnitsky, A. Leavitt, M. J. Schwaber, D. Schwartz, and Y. Carmeli.** 2006. Plasmid-mediated imipenem-hydrolyzing enzyme KPC-2 among multiple carbapenem-resistant *Escherichia coli* clones in Israel. Antimicrob. Agents Chemother. **50:**3098–3101.

148. **Nishio, H., M. Komatsu, N. Shibata, K. Shimakawa, N. Sueyoshi, T. Ura, K. Satoh, M. Toyokawa, T. Nakamura, Y. Wada, T. Orita, T. Kofuku, K. Yamasaki, M. Sakamoto, S. Kinoshita, M. Aihara, and Y. Arakawa.** 2004. Metallo-β-lactamase-producing gram-negative bacilli: laboratory-based surveillance in cooperation with 13 clinical laboratories in the Kinki region of Japan. J. Clin. Microbiol. **42:**5256–5263.

149. **Nordmann, P., S. Mariotte, T. Naas, R. Labia, and M.-H. Nicolas.** 1993. Biochemical properties of a carbapenem-hydrolyzing β-lactamase for *Enterobacter cloacae* and cloning of the gene into *Escherichia coli*. Antimicrob. Agents Chemother. **37:**939–946.

150. **Nordmann, P., and L. Poirel.** 2002. Emerging carbapenemases in Gram-negative aerobes. Clin. Microbiol. Infect. **8:**321–331.

151. **Normark, S.** 1995. β-Lactamase induction in gram-negative bacteria is intimately linked to peptidoglycan recycling. Microb. Drug Resist. Mech. Epidemiol. Dis. **1:**111–114.

152. **O'Hara, K., S. Haruta, T. Sawai, M. Tsunoda, and S. Iyobe.** 1998. Novel metallo β-lactamase mediated by a *Shigella flexneri* plasmid. FEMS Microbiol. Lett. **162:**201–206.

153. **Paetzel, M., F. Danel, L. de Castro, S. C. Mosimann, M. G. Page, and N. C. Strynadka.** 2000. Crystal structure of the class D β-lactamase OXA-10. Nat. Struct. Biol. **7:**918–925.

154. **Pagani, L., C. Colinon, R. Migliavacca, M. Labonia, J.-D. Docquier, E. Nucleo, M. Spalla, M. Li Bergoli, and G. M. Rossolini.** 2005. Nosocomial outbreak caused by multidrug-resistant *Pseudomonas aeruginosa* producing IMP-13 metallo-β-lactamase. J. Clin. Microbiol. **43:**3824–3828.

155. **Pasteran, F., D. Faccone, A. Petroni, M. Rapoport, M. Galas, M. Vazquez, and A. Procopio.** 2005. Novel variant (*bla*_{VIM-11}) of the metallo-β-lactamase *bla*_{VIM} family in a GES-1 extended-spectrum-β-lactamase-producing *Pseudomonas aeruginosa* clinical isolate in Argentina. Antimicrob. Agents Chemother. **49:**474–475.

156. **Paterson, D. L., and R. A. Bonomo.** 2005. Extended-spectrum β-lactamases: a clinical update. Clin. Microbiol. Rev. **18:**657–686.

157. **Paton, R., R. S. Miles, J. Hood, and S. G. B. Amyes.** 1993. ARI 1: β-lactamase-mediated imipenem resistance in *Acinetobacter baumannii*. Int. J. Antimicrob. Agents **2:**81–87.

158. **Peleg, A. Y., C. Frankiin, J. M. Bell, and D. W. Spelman.** 2005. Dissemination of the metallo-β-lactamase gene bla_{IMP-4} among Gram-negative pathogens in a clinical setting in Australia. Clin. Infect. Dis. **41:**1549–1556.

159. **Peleg, A. Y., C. Franklin, J. Bell, and D. W. Spelman.** 2004. Emergence of IMP-4 metallo-β-lactamase in a clinical isolate from Australia. J. Antimicrob. Chemother. **54:**699–700.

160. **Peleg, A. Y., C. Franklin, L. J. Walters, J. M. Bell, and D. W. Spelman.** 2006. OXA-58 and IMP-4 carbapenem-hydrolyzing β-lactamases in an *Acinetobacter junii* blood culture isolate from Australia. Antimicrob. Agents Chemother. **50:**399–400.

161. **Petropoulou, D., K. Tzanetou, V. P. Syriopoulou, G. L. Daikos, G. Ganteris, and E. Malamou-Lada.** 2006. Evaluation of imipenem/imipenem + EDTA disk method for detection of metallo-β-lactamase-producing *Klebsiella pneumoniae* isolated from blood cultures. Microb. Drug Resist. **12:**39–43.

162. **Philippon, L. N., T. Naas, A.-T. Bouthors, V. Barakett, and P. Nordmann.** 1997. OXA-18, a class D clavulanic acid-inhibited extended-spectrum β-lactamase from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **41:**2188–2195.

163. **Pitout, J. D. D., B. L. Chow, D. B. Gregson, K. B. Laupland, S. Elsayed, and D. L. Church.** 2007. Molecular epidemiology of metallo-β-lactamase-producing *Pseudomonas aeruginosa* in the Calgary Health Region: emergence of VIM-2-producing isolates. J. Clin. Microbiol. **45:**294–298.

164. **Poirel, L., L. Brinas, N. Fortineau, and P. Nordmann.** 2005. Integron-encoded GES-type extended-spectrum β-lactamase with increased activity toward aztreonam in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **49:**3593–3597.

165. **Poirel, L., C. Heritier, and P. Nordmann.** 2004. Chromosome-encoded Ambler class D β-lactamase of *Shewanella oneidensis* as a progenitor of carbapenem-hydrolyzing oxacillinase. Antimicrob. Agents Chemother. **48:**348–351.

166. **Poirel, L., C. Heritier, and P. Nordmann.** 2005. Genetic and biochemical characterization of the chromosome-encoded class B β-lactamases from *Shewanella livingstonensis* (SLB-1) and *Shewanella frigidimarina* (SFB-1). J. Antimicrob. Chemother. **55:**680–685.

167. **Poirel, L., C. Heritier, V. Toluen, and P. Nordmann.** 2004. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **48:**15–22.

168. **Poirel, L., I. Le Thomas, T. Naas, A. Karim, and P. Nordmann.** 2000. Biochemical sequence analyses of GES-1, a novel class A extended-spectrum β-lactamase, and the class 1 integron In52 from *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **44:**622–632.

169. **Poirel, L., M. Magalhaes, M. Lopes, and P. Nordmann.** 2004. Molecular analysis of metallo-β-lactamase gene *bla*_{SPM-1}-surrounding sequences from disseminated *Pseudomonas aeruginosa* isolates in Recife, Brazil. Antimicrob. Agents Chemother. **48:**1406–1409.

170. **Poirel, L., S. Marque, C. Heritier, C. Segonds, G. Chabanon, and P. Nordmann.** 2005. OXA-58, a novel class D β-lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **49:**202–208.

171. **Poirel, L., T. Naas, D. Nicolas, L. Collet, S. Bellais, J.-D. Cavallo, and P. Nordmann.** 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob. Agents Chemother. **44:**891–897.

172. **Poirel, L., J. N. Pham, L. Cabanne, B. J. Gatus, S. M. Bell, and P. Nordmann.** 2004. Carbapenem-hydrolysing metallo-β-lactamases from *Klebsiella pneumoniae* and *Escherichia coli* isolated in Australia. Pathology (Philadelphia) **36:**366–367.

173. **Poirel, L., G. F. Weldhagen, C. De Champs, and P. Nordmann.** 2002. A nosocomial outbreak of *Pseudomonas aeruginosa* isolates expressing the extended-spectrum β-lactamase GES-2 in South Africa. J. Antimicrob. Chemother. **49:**561–565.

174. **Poirel, L., G. F. Weldhagen, T. Naas, C. De Champs, M. G. Dove, and P. Nordmann.** 2001. GES-2, a class A β-lactamase from *Pseudomonas aeruginosa* with increased hydrolysis of imipenem. Antimicrob. Agents Chemother. **45:**2598–2603.

175. **Pottumarthy, S., E. S. Moland, S. Jeretschko, S. R. Swanzy, K. S. Thomson, and T. R. Fritsche.** 2003. NmcA carbapenem-hydrolyzing enzyme in *Enterobacter cloacae* in North America. Emerg. Infect. Dis. **9:**999–1002.

176. **Quale, J., S. Bratu, J. Gupta, and D. Landman.** 2006. Interplay of efflux system, *ampC*, and *oprD* expression in carbapenem resistance of *Pseudomonas aeruginosa* clinical isolates. Antimicrob. Agents Chemother. **50:**1633–1641.

177. **Quale, J. M., D. Landman, P. A. Bradford, M. Visalli, J. Ravishankar, C. Flores, D. Mayorga, K. Vangala, and A. Adedeji.** 2002. Molecular epidemiology of a citywide outbreak of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae infection. Clin. Infect. Dis. **35:**834–841.

178. **Queenan, A. M., W. Shang, P. Schreckenberger, K. Lolans, K. Bush, and J. Quinn.** SME-3, a novel member of the *Serratia marcescens* SME family of carbapenem-hydrolyzing β-lactamases. Antimicrob. Agents Chemother. **50:**3485–3487.

179. **Queenan, A. M., C. Torres-Viera, H. S. Gold, Y. Carmeli, G. M. Eliopoulos, R. C. Moellering, Jr., J. P. Quinn, J. Hindler, A. A. Medeiros, and K. Bush.** 2000. SME-type carbapenem-hydrolyzing class A β-lactamases from geographically diverse *Serratia marcescens* strains. Antimicrob. Agents Chemother. **44:**3035–3039.

180. **Quinteira, S., and H. F. Luisa-Peixe.** 2005. First isolation of *bla*~VIM-2~ in an environmental isolate of *Pseudomonas pseudoalcaligenes*. Antimicrob. Agents Chemother. **49:**2140–2141.

181. **Radice, M., P. Power, G. Gutkind, K. Fernandez, C. Vay, A. Famiglietti, N. Ricover, and J. Ayala.** 2004. First class A carbapenemase isolated from *Enterobacteriaceae* in Argentina. Antimicrob. Agents Chemother. **48:**1068–1069.

182. **Rasmussen, B. A., and K. Bush.** 1997. Carbapenem-hydrolyzing β-lactamases. Antimicrob. Agents Chemother. **41:**223–232.

183. **Rasmussen, B. A., K. Bush, D. Keeney, Y. Yang, R. Hare, C. O'Gara, and A. A. Medeiros.** 1996. Characterization of IMI-1 β-lactamase, a class A carbapenem-hydrolyzing enzyme from *Enterobacter cloacae*. Antimicrob. Agents Chemother. **40:**2080–2086.

184. **Riccio, M. L., N. Franceschini, L. Boschi, B. Caravelli, G. Cornaglia, R. Fontana, G. Amicosante, and G. M. Rossolini.** 2000. Characterization of the metallo-β-lactamase determinant of *Acinetobacter baumannii* AC-54/97 reveals the existence of *bla*~IMP~ allelic variants carried by gene cassettes of different phylogeny. Antimicrob. Agents Chemother. **44:**1229–1235.

185. **Richmond, M. H., and R. B. Sykes.** 1973. The β-lactamases of Gram-negative bacteria and their possible physiological role, p. 31–88. *In* A. H. Rose and D. W. Tempest (ed.), Advances in microbial physiology, vol. 9. Academic Press, New York, NY.

186. **Ryoo, N. H., E.-C. Kim, S. G. Hong, Y. J. Park, K. Lee, I. K. Bae, E. H. Song, and S. H. Jeong.** 2005. Dissemination of SHV-12 and CTX-M-type extended-spectrum β-lactamases among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* and emergence of GES-3 in Korea. J. Antimicrob. Chemother. **56:**698–702.

187. **Sader, H. S., A. O. Reis, S. Silbert, and A. C. Gales.** 2005. IMPs, VIMs and SPMs: the diversity of metallo-β-lactamases produced by carbapenem-resistant *Pseudomonas aeruginosa* in a Brazilian hospital. Clin. Microbiol. Infect. **11:**73–76.

188. **Saino, Y., F. Kobayashi, M. Inoue, and S. Mitsuhashi.** 1982. Purification and properties of inducible penicillin β-lactamase isolated from *Pseudomonas maltophilia*. Antimicrob. Agents Chemother. **22:**564–570.

189. **Santillana, E., A. Beceiro, G. Bou, and A. Romero.** 2007. Crystal structure of the carbapenemase OXA-24 reveals insights into the mechanism of carbapenem hydrolysis. Proc. Natl. Acad. Sci. USA **104:**5354–5359.

190. **Sardelic, S., L. Pallecchi, V. Punda-Polic, and G. M. Rossolini.** 2003. Carbapenem-resistant *Pseudomonas aeruginosa*-carrying VIM-2 metallo-β-lactamase determinants, Croatia. Emerg. Infect. Dis. **9:**1022–1023.

191. **Scaife, W., H.-K. Young, R. H. Paton, and S. G. B. Amyes.** 1995. Transferable imipenem-resistance in *Acinetobacter* species from a clinical source. J. Antimicrob. Chemother. **36:**585–586.

192. **Schneider, I., A. M. Queenan, and A. Bauernfeind.** 2006. Novel carbapenem-hydrolyzing oxacillinase OXA-62 from *Pandoraea pnomenusa*. Antimicrob. Agents Chemother. **50:**1330–1335.

193. **Scoulica, E. V., I. K. Neonakis, A. I. Gikas, and Y. J. Tselentis.** 2004. Spread of *bla*~VIM-1~-producing *E. coli* in a university hospital in Greece. Genetic analysis of the integron carrying the *bla*~VIM-1~ metallo-β-lactamase gene. Diagn. Microbiol. Infect. Dis. **48:**167–172.

194. **Segal, H., and B. G. Elisha.** 2005. Use of Etest MBL strips for the detection of carbapenemases in *Acinetobacter baumannii*. J. Antimicrob. Chemother. **56:**598.

195. **Senda, K., Y. Arakawa, S. Ichiyama, K. Nakashima, H. Ito, S. Ohsuka, K. Shimokata, N. Kato, and M. Ohta.** 1996. PCR detection of metallo-β-lactamase gene (*bla*~IMP~) in gram-negative rods resistant to broad-spectrum β-lactams. J. Clin. Microbiol. **34:**2909–2913.

196. **Shannon, K., A. King, and I. Phillips.** 1986. β-Lactamases with high activity against imipenem and Sch 34343 from *Aeromonas hydrophila*. J. Antimicrob. Chemother. **17:**45–50.

197. **Shibata, N., Y. Doi, K. Yamane, T. Yagi, H. Kurokawa, K. Shibayama, H. Kato, K. Kai, and Y. Arakawa.** 2003. PCR typing of genetic determinants for metallo-β-lactamases and integrases carried by gram-negative bacteria isolated in Japan, with focus on the class 3 integron. J. Clin. Microbiol. **41:**5407–5413.

198. **Simpson, I. N., P. B. Harper, and C. H. O'Callaghan.** 1980. Principal β-lactamases responsible for resistance to β-lactam antibiotics in urinary tract infections. Antimicrob. Agents Chemother. **17:**929–936.

199. **Sougakoff, W., G. L'Hermite, L. Pernot, T. Naas, V. Guillet, P. Nordmann, V. Jarlier, and J. Delettre.** 2002. Structure of the imipenem-hydrolyzing class A β-lactamase SME-1 from *Serratia marcescens*. Acta Crystallogr. D **58:**267–274.

200. **Sougakoff, W., T. Naas, P. Nordmann, E. Collatz, and V. Jarlier.** 1999. Role of Ser-237 in the substrate specificity of the carbapenem-hydrolyzing class A β-lactamase Sme-1. Biochim. Biophys. Acta **1433:**153–158.

201. **Swaren, P., L. Maveyraud, X. Raquet, S. Cabantous, C. Duez, J.-D. Pedelacq, S. Mariotte-Boyer, L. Mourey, R. Labia, M.-H. Nicolas-Chanoine, P. Nordmann, J.-M. Frere, and J.-P. Samama.** 1998. X-ray analysis of the NMC-A β-lactamase at 1.64-Å resolution, a class A carbapenemase with broad substrate specificity. J. Biol. Chem. **273:**26714–26721.

202. **Sykes, R. B., and M. Matthew.** 1976. The β-lactamases of Gram-negative bacteria and their role in resistance to β-lactam antibiotics. J. Antimicrob. Chemother. **2:**115–157.

203. **Tenover, F. C., R. K. Kalsi, P. P. Williams, R. B. Carey, S. Stocker, D. Lonsway, J. K. Rasheed, J. W. Biddle, J. E. McGowan, Jr., and B. Hanna.** 2006. Carbapenem resistance in *Klebsiella pneumoniae* not detected by automated susceptibility testing. Emerg. Infect. Dis. **12:**1209–1213.

204. **Toleman, M. A., K. Rolston, R. N. Jones, and T. R. Walsh.** 2004. *bla*~VIM-7~, an evolutionarily distinct metallo-β-lactamase gene in a *Pseudomonas aeruginosa* isolate from the United States. Antimicrob. Agents Chemother. **48:**329–332.

205. **Toleman, M. A., K. Rolston, R. N. Jones, and T. R. Walsh.** 2003. Molecular and biochemical characterization of OXA-45, an extended-spectrum class 2d′ β-lactamase in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **47:**2859–2863.

206. **Toleman, M. A., A. M. Simm, T. A. Murphy, A. C. Gales, D. J. Biedenbach, R. N. Jones, and T. R. Walsh.** 2002. Molecular characterization of SPM-1, a novel metallo-β-lactamase isolated in Latin America: report from the SENTRY antimicrobial surveillance programme. J. Antimicrob. Chemother. **50:**673–679.

207. **Tortola, M. T., S. Lavilla, E. Miro, J. J. Gonzalez, N. Larrosa, M. Sabate, F. Navarro, and G. Prats.** 2005. First detection of a carbapenem-hydrolyzing metalloenzyme in two *Enterobacteriaceae* isolates in Spain. Antimicrob. Agents Chemother. **49:**3492–3494.

208. **Troillet, N., Y. Carmeli, L. Venkataraman, P. DeGirolami, and M. H. Samore.** 1999. Epidemiological analysis of imipenem-resistant *Serratia marcescens* in hospitalized patients. J. Hosp. Infect. **42:**37–43.

209. **Turner, P. J.** 2004. Susceptibility of meropenem and comparators tested against 30,634 Enterobacteriaceae isolated in the MYSTIC Programme (1997–2003). Diagn. Microbiol. Infect. Dis. **50:**291–293.

210. **Turner, P. J.** 2005. Trends in antimicrobial susceptibilities among bacterial pathogens isolated from patients hospitalized in European medical centers: 6-year report of the MYSTIC Surveillance Study (1997–2002). Diagn. Microbiol. Infect. Dis. **51:**281–289.

211. **Turton, J. F., M. E. Kaufmann, J. Glover, J. M. Coelho, M. Warner, R. Pike, and T. L. Pitt.** 2005. Detection and typing of integrons in epidemic strains of *Acinetobacter baumannii* found in the United Kingdom. J. Clin. Microbiol. **43:**3074–3082.

212. **Turton, J. F., M. E. Ward, N. Woodford, M. E. Kaufmann, R. Pike, D. M. Livermore, and T. L. Pitt.** 2006. The role of ISAba1 in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. FEMS Microbiol. Lett. **258:**72–77.

213. **Turton, J. F., N. Woodford, J. Glover, S. Yarde, M. E. Kaufmann, and T. L. Pitt.** 2006. Identification of *Acinetobacter baumannii* by detection of the *bla*~OXA-51~-like carbapenemase gene intrinsic to this species. J. Clin. Microbiol. **44:**2974–2976.

214. **Ullah, J. H., T. R. Walsh, I. A. Taylor, D. C. Emery, C. S. Verma, S. J. Gamblin, and J. Spencer.** 1998. The crystal structure of the L1 metallo-β-lactamase from *Stenotrophomonas maltophilia* at 1.7 Å resolution. J. Mol. Biol. **284:**125–136.

215. **Vahaboglu, H., F. Budak, M. Kasap, G. Gacar, S. Torol, A. Karadenizli, F. Kolayli, and C. Eroglu.** 2006. High prevalence of OXA-51-type class D β-lactamases among ceftazidime-resistant clinical isolates of *Acinetobacter* spp.: co-existence with OXA-58 in multiple centres. J. Antimicrob. Chemother. **58:**537–542.

216. **Villegas, M. V., K. Lolans, A. Correa, J. N. Kattan, J. A. Lopez, J. P. Quinn, and the Colombian Nosocomial Resistance Study Group.** 2007. First identification of *Pseudomonas aeruginosa* isolates producing a KPC-type carbapenem-hydrolyzing β-lactamase. Antimicrob. Agents Chemother. **51:**1553–1555.

217. **Villegas, M. V., K. Lolans, O. M. del Rosario, C. J. Suarez, A. Correa, A. M. Queenan, and J. P. Quinn.** 2006. First detection of metallo-β-lactamase VIM-2 in *Pseudomonas aeruginosa* isolates from Colombia. Antimicrob. Agents Chemother. **50:**226–229.

218. **Vourli, S., P. Giakkoupi, V. Miriagou, E. Tzelepi, A. C. Vatopoulos, and L. S. Tzouvelekis.** 2004. Novel GES/IBC extended-spectrum β-lactamase variants with carbapenemase activity in clinical Enterobacteria. FEMS Microbiol. Lett. **234:**209–213.
219. **Wachino, J.-I., Y. Doi, K. Yamane, N. Shibata, T. Yagi, T. Kubota, and Y. Arakawa.** 2004. Molecular characterization of a cephamycin-hydrolyzing and inhibitor-resistant class A β-lactamase, GES-4, possessing a single G170S substitution in the omega-loop. Antimicrob. Agents Chemother. **48:**2905–2910.
220. **Wachino, J.-I., Y. Doi, K. Yamane, N. Shibata, T. Yagi, T. Kubota, H. Ito, and Y. Arakawa.** 2004. Nosocomial spread of ceftazidime-resistant *Klebsiella pneumoniae* strains producing a novel class A β-lactamase, GES-3, in a neonatal intensive care unit in Japan. Antimicrob. Agents Chemother. **48:**1960–1967.
221. **Walsh, T. R.** 2005. The emergence and implications of metallo-β-lactamases in Gram-negative bacteria. Clin. Microbiol. Infect. **11:**2–9.
222. **Walsh, T. R., A. Bolmstrom, A. Qwarnstrom, and A. Gales.** 2002. Evaluation of a new Etest for detecting metallo-β-lactamases in routine clinical testing. J. Clin. Microbiol. **40:**2755–2759.
223. **Walsh, T. R., D. J. Payne, A. P. MacGowan, and P. M. Bennett.** 1995. A clinical isolate of *Aeromonas sobria* with three chromosomally mediated inducible β-lactamases: a cephalosporinase, a penicillinase and a third enzyme displaying carbapenemase activity. J. Antimicrob. Chemother. **37:**423–431.
224. **Walsh, T. R., M. A. Toleman, L. Poirel, and P. Nordmann.** 2005. Metallo-β-lactamases: the quiet before the storm? Clin. Microbiol. Rev. **18:**306–325.
225. **Walther-Rasmussen, J., and N. Hoiby.** 2006. OXA-type carbapenemases. J. Antimicrob. Chemother. **57:**373–383.
226. **Wang, C.-X., and Z.-H. Mi.** 2004. IMP-1 metallo-β-lactamase-producing *Pseudomonas aeruginosa* in a university hospital in the People's Republic of China. J. Antimicrob. Chemother. **54:**1159–1160.
227. **Wang, C., J. Wang, and Z. Mi.** 2006. *Pseudomonas aeruginosa* producing VIM-2 metallo-β-lactamases and carrying two aminoglycoside-modifying enzymes in China. J. Hosp. Infect. **62:**522–524.
228. **Watanabe, M., S. Iyobe, M. Inoue, and S. Mitsuhashi.** 1991. Transferable imipenem resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **35:**147–151.
229. **Wei, Z.-Q., X.-X. Du, Y.-S. Yu, P. Shen, Y.-G. Chen, and L.-J. Li.** 2007. Plasmid-mediated KPC-2 in a *Klebsiella pneumoniae* isolate from China. Antimicrob. Agents Chemother. **51:**763–765.
230. **Weldhagen, G. F., and A. Prinsloo.** 2004. Molecular detection of GES-2 extended spectrum β-lactamase producing *Pseudomonas aeruginosa* in Pretoria, South Africa. Int. J. Antimicrob. Agents **24:**35–38.
231. **Reference deleted.**
232. **Woodford, N., M. J. Ellington, J. M. Coelho, J. F. Turton, M. E. Ward, S. Brown, S. G. B. Amyes, and D. M. Livermore.** 2006. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int. J. Antimicrob. Agents **27:**351–353.
233. **Woodford, N., P. M. Tierno, Jr., K. Young, L. Tysall, M.-F. I. Palepou, E. Ward, R. E. Painter, D. F. Suber, D. Shungu, L. L. Silver, K. Inglima, J. Kornblum, and D. M. Livermore.** 2004. Outbreak of *Klebsiella pneumoniae* producing a new carbapenem-hydrolyzing class A β-lactamase, KPC-3, in a New York medical center. Antimicrob. Agents Chemother. **48:**4793–4799.
234. **Xiong, J., M. F. Hynes, H. Ye, H. Chen, Y. Yang, F. M'Zali, and P. M. Hawkey.** 2006. *bla*_{IMP-9} and its association with large plasmids carried by *Pseudomonas aeruginosa* isolates from the People's Republic of China. Antimicrob. Agents Chemother. **50:**355–358.
235. **Yamasaki, K., M. Komatsu, T. Yamashita, K. Shimakawa, T. Ura, H. Nishio, K. Satoh, R. Washidu, S. Kinoshita, and M. Aihara.** 2003. Production of CTX-M-3 extended-spectrum β-lactamase and IMP-1 metallo β-lactamase by five Gram-negative bacilli: survey of clinical isolates from seven laboratories collected in 1998 and 2000, in the Kinki region of Japan. J. Antimicrob. Chemother. **51:**631–638.
236. **Yan, J.-J., P.-R. Hsueh, J.-J. Lu, F.-Y. Chang, W.-C. Ko, and J.-J. Wu.** 2006. Characterization of acquired β-lactamases and their genetic support in multidrug-resistant *Pseudomonas aeruginosa* isolates in Taiwan: the prevalence of unusual integrons. J. Antimicrob. Chemother. **58:**530–536.
237. **Yan, J.-J., W.-C. Ko, C.-L. Chuang, and J.-J. Wu.** 2002. Metallo-β-lactamase-producing *Enterobacteriaceae* isolates in a university hospital in Taiwan: prevalence of IMP-8 in *Enterobacter cloacae* and first identification of VIM-2 in *Citrobacter freundii*. J. Antimicrob. Chemother. **50:**503–511.
238. **Yan, J.-J., W.-C. Ko, and J.-J. Wu.** 2001. Identification of a plasmid encoding SHV-12, TEM-1, and a variant of IMP-2 metallo-β-lactamase, IMP-8, from a clinical isolate of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **45:**2368–2371.
239. **Yan, J.-J., J.-J. Wu, S.-H. Tsai, and C.-L. Chuang.** 2004. Comparison of the double-disk, combined disk, and Etest methods for detecting metallo-β-lactamases in gram-negative bacilli. Diagn. Microbiol. Infect. Dis. **49:**5–11.
240. **Yan, J. J., P. R. Hsueh, W. C. Ko, K. T. Luh, S. H. Tsai, H. M. Wu, and J. J. Wu.** 2001. Metallo-β-lactamases in clinical *Pseudomonas* isolates in Taiwan and identification of VIM-3, a novel variant of the VIM-2 enzyme. Antimicrob. Agents Chemother. **45:**2224–2228.
241. **Yang, Y., and K. Bush.** 1996. Biochemical characterization of the carbapenem-hydrolyzing β-lactamase AsbM1 from *Aeromonas sobria* AER 14M: a member of a novel subgroup of metallo-β-lactamases. FEMS Microbiol. Lett. **137:**193–200.
242. **Yang, Y., B. A. Rasmussen, and K. Bush.** 1992. Biochemical characterization of the metallo-β-lactamase CcrA from *Bacteroides fragilis* TAL3636. Antimicrob. Agents Chemother. **36:**1155–1157.
243. **Yang, Y., P. Wu, and D. M. Livermore.** 1990. Biochemical characterization of a β-lactamase that hydrolyzes penems and carbapenems from two *Serratia marcescens* isolates. Antimicrob. Agents Chemother. **34:**755–758.
244. **Yano, H., A. Kuga, R. Okamoto, H. Kitasato, T. Kobayashi, and M. Inoue.** 2001. Plasmid-encoded metallo-β-lactamase (IMP-6) conferring resistance to carbapenems, especially meropenem. Antimicrob. Agents Chemother. **45:**1343–1348.
245. **Yigit, H., A. M. Queenan, G. J. Anderson, A. Domenech-Sanchez, J. W. Biddle, C. D. Steward, S. Alberti, K. Bush, and F. C. Tenover.** 2001. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **45:**1151–1161.
246. **Yigit, H., A. M. Queenan, K. Rasheed, J. W. Biddle, A. Domenech-Sanchez, S. Alberti, K. Bush, and F. C. Tenover.** 2003. Carbapenem-resistant strain of *Klebsiella oxytoca* harboring carbapenem-hydrolyzing β-lactamase KPC-2. Antimicrob. Agents Chemother. **47:**3881–3889.
247. **Yong, D., S. Choi Yeoung, H. Roh Kyoung, K. Kim Chang, H. Park Youn, H. Yum Jong, K. Lee, and Y. Chong.** 2006. Increasing prevalence and diversity of metallo-β-lactamases in *Pseudomonas* spp., *Acinetobacter* spp., and *Enterobacteriaceae* from Korea. Antimicrob. Agents Chemother. **50:**1884–1886.
248. **Yong, D., K. Lee, J. H. Yum, H. B. Shin, G. M. Rossolini, and Y. Chong.** 2002. Imipenem-EDTA disk method for differentiation of metallo-β-lactamase-producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. J. Clin. Microbiol. **40:**3798–3801.
249. **Yu, Y.-S., X.-X. Du, Z.-H. Zhou, Y.-G. Chen, and L.-J. Li.** 2006. First isolation of *bla*_{IMI-2} in an *Enterobacter cloacae* clinical isolate from China. Antimicrob. Agents Chemother. **50:**1610–1611.
250. **Yum, J. H., D. Yong, K. Lee, H.-S. Kim, and Y. Chong.** 2002. A new integron carrying VIM-2 metallo-β-lactamase gene cassette in a *Serratia marcescens* isolate. Diagn. Microbiol. Infect. Dis. **42:**217–219.
251. **Zavascki, A. P., P. B. Gaspareto, A. F. Martins, A. L. Goncalves, and A. L. Barth.** 2005. Outbreak of carbapenem-resistant *Pseudomonas aeruginosa* producing SPM-1 metallo-β-lactamase in a teaching hospital in southern Brazil. J. Antimicrob. Chemother. **56:**1148–1151.